Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Table S1 Composition of amino acid mixture solution CRM

Amino acids	Certified Value（mmol/L）	Uncertainty (mmol/L)，k=2
Aspartate	0.991	0.03
Threonine	0.991	0.03
Serine	0.994	0.03
Glutamate	0.982	0.03
Glycine	0.988	0.03
Alanine	0.993	0.03
Cystine	0.991	0.04
Valine	0.993	0.04
Methionine	0.999	0.03
Isoleucine	0.997	0.02
Leucine	0.991	0.04
Tyrosine	0.994	0.03
Phenylalanine	0.994	0.03
Lysine hydrochloride	0.994	0.02
Histidine	0.995	0.04
Arginine	0.992	0.03
Proline	0.995	0.02

Uncertainty Evaluation

Firstly, the uncertainty of mass fraction of [Glu[1]]-fibrinopeptide B determined by GC-IDMS without impurity correction was evaluated and shown in Table S2.

Table S2 Uncertainty evaluation of [Glu[1]]-fibrinopeptide B mass fraction determined by GC-IDMS (g/g)

Uncertainty source	Uncertainty u_i	Sensitivity factor c_i	$c_i \times u_i$
Weighing of Val CRM	5.77E-07	64.3997	3.72E-05
Weighing of Phe CRM	5.77E-07	42.5346	2.46E-05
Val purity	0.003	0.3625	0.0010875
Phe purity	0.002	0.3732	0.0007464
Weighing of 0.1 mol/L HCl for Val dissolving	5.77E-06	-0.0647	-3.73E-07
Weighing of 0.1 mol/L HCl for Phe dissolving	5.77E-06	-0.0426	-2.46E-07
Weighing of Val stock solution	5.77E-06	0.5759	3.32E-06
Weighing of Phe stock solution	5.77E-06	0.1167	6.74E-07
Weighing of 0.1 mol/L HCl for AA working solution	5.77E-06	-0.1493	-8.61984E-07

Weighing of L-Val	5.77E-07	0.0000	0
Weighing of L-Phe	5.77E-07	0.0000	0
Weighing of 0.1 mol/L HCl for L-Val dissolving	5.77E-06	0.0000	0
Weighing of 0.1 mol/L HCl for L-Phe dissolving	5.77E-06	0.0000	0
Weighing of L-Val stock solution	5.77E-06	0.0000	0
Weighing of L-Phe stock solution	5.77E-06	0.0000	0
Weighing of 0.1 mol/L HCl for L-AA working solution	5.77E-06	0.0000	0
Weighing of AA working solution for calibrator	5.77E-06	21.4966	1.24E-04
Weighing of L-AA working solution for calibrator	5.77E-06	-15.0507	-8.69E-05
Weighing of L-AA working solution for sample	5.77E-06	15.0817	8.70742E-05

Peak area ratio of Val of calibrator	0	-0.4910	0
Peak area ratio of Phe of calibrator	0	-0.4400	0
Peak area ratio of Val of sample	0	0.4928	0
Peak area ratio of Phe of sample	0	0.4272	0
Weighing of sample	5.77E-07	-331.2639	-1.91E-04
Weighing of 0.1%TFA for sample dissolving	5.77E-06	0.3292	1.90064E-06
Weighing of sample for hydrolysis	5.77E-06	-11.4235	-6.59536E-05
Molecular weight of Val	0.0018544	-0.0031	-5.71155E-06
Molecular weight of Phe	0.002449245	-0.0023	-5.52795E-06
Molecular weight of [Glu^{1}]-fibrinopeptide B	0.00672	0.0005	3.1369E-06
Number of Val in [Glu^{1}]-fibrinopeptide B	0	-0.3604	0

Number of Phe in [Glu^1]-fibrinopeptide B	0	-0.1862	0
Method repeatability	1.17E-03	1.0000	1.17E-03
Variation between Val and Phe	5.58E-03	1.0000	5.58E-03
Combined standard uncertainty (u_x)			0.005861639

The [Glu^1]-fibrinopeptide B purity is calculated by formula S.1:

$$c = xP \quad \text{S.1}$$

where,

c—— [Glu^1]-fibrinopeptide B purity， g/g

x—— mass fraction of [Glu^1]-fibrinopeptide B, g/g

P—— relative peak area ratio of [Glu^1]-fibrinopeptide B in total peaks determined by LC-UV.

Therefore, the uncertainty of c can be estimated by formula (S.2):

$$\frac{u_c}{c} = \sqrt{(\frac{u_P}{P})^2 + (\frac{u_x}{x})^2} \quad \text{S.2}$$

$$u_c = c\sqrt{(\frac{u_P}{P})^2 + (\frac{u_x}{x})^2} \quad \text{S.3}$$

$$= 0.715 \times \sqrt{(\frac{0.1016}{96.77})^2 + (\frac{0.0059}{0.739})^2} = 0.00576$$

$$U = ku_c = 2 \times 0.00576 = 0.012 \quad g/g \qquad \text{S.4}$$

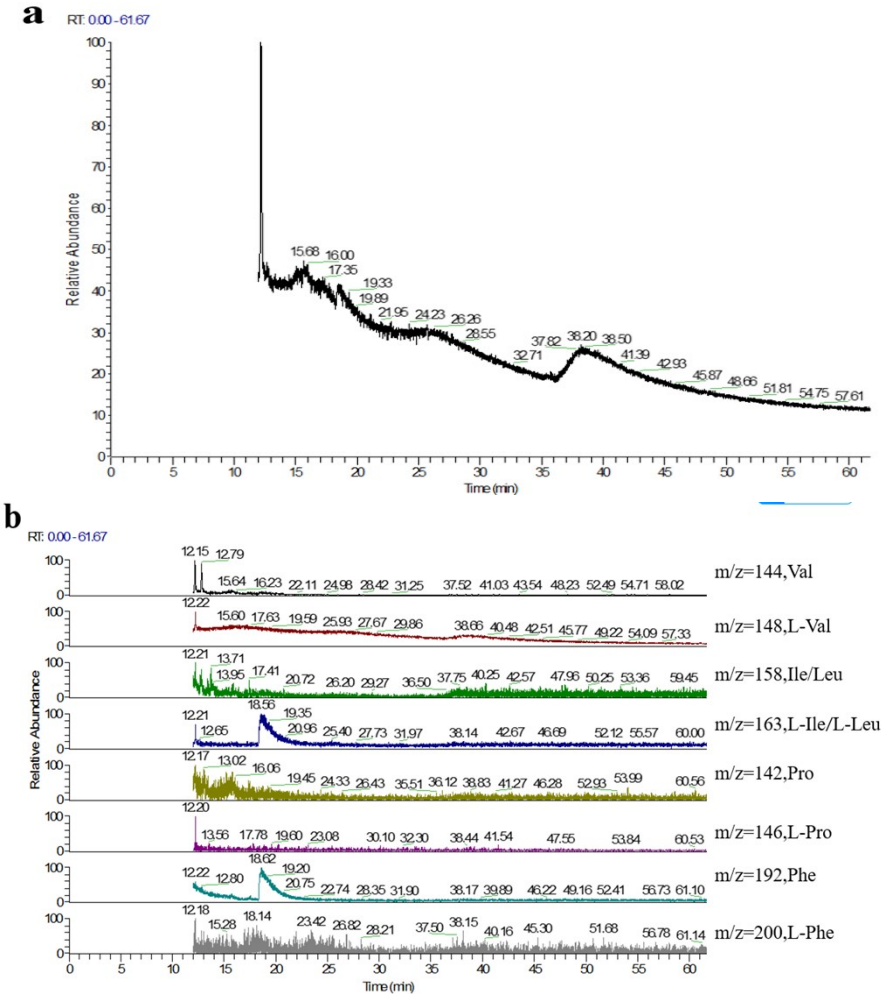

Figure S1 GC-MS chromatogram of blank

(a is the TIC and b is the EIC for 5 amino acids with their isotopic analogues)

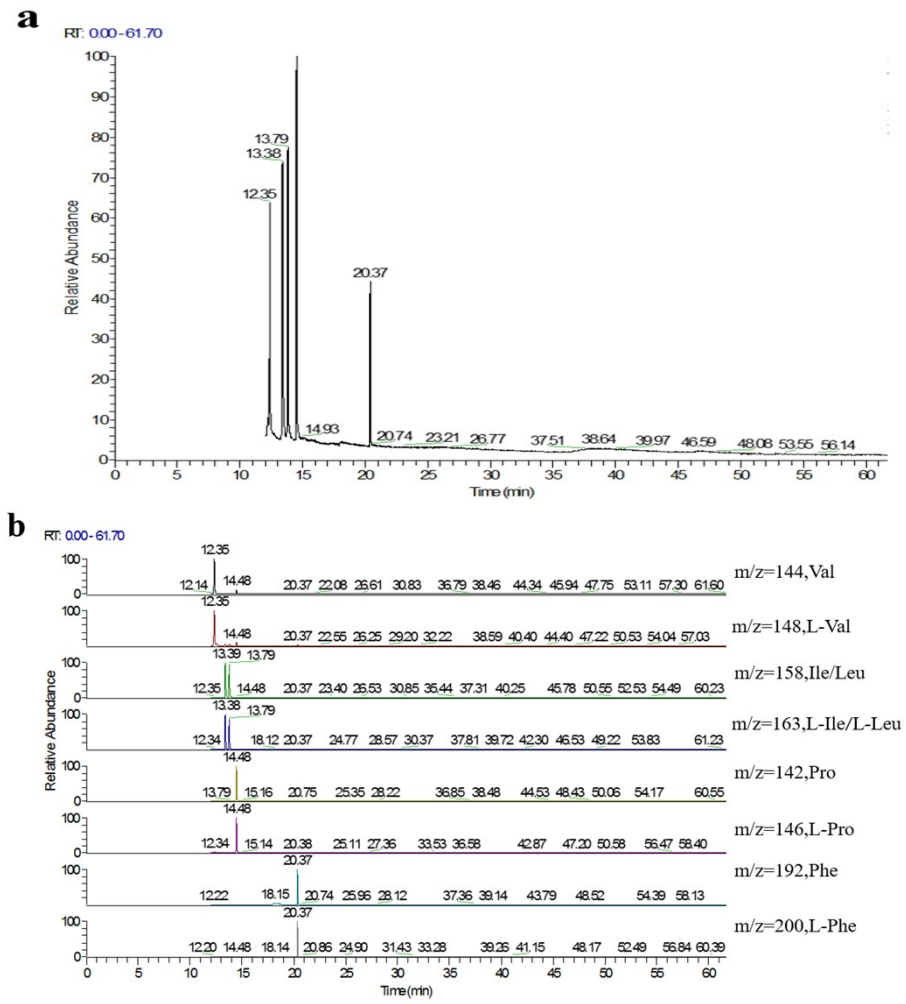

Figure S2 GC-MS chromatogram of amino acid calibration blend

(a is the TIC of amino acid calibration blend, b is the EIC of 5 amino acids with their isotopic analogues)