Electronic Supplementary Material (ESI) for Analytical Methods.

1. Solution preparation

Mixed amino-acid standard work solutions for hCG hydrolysis quantification: A mixed label-free amino-acid standard solution (MLFS) contained 102 μL phenylalanine stock solutions, 278 μL proline stock solutions, 191 μL valine stock solution and 9429 μL water. A mixed isotope labeled solution (MILS) contained 102 μL $^{13}C_9$-L-phenylalanine stock solution, 278 μL $^{13}C_5$ L-proline stock solution, 278 μL $^{13}C_5$-L-valine stock solution and 9342 μL water. A lower level standard solution contained 123 μL MLFS and 98 μL MILS. A higher level standard solution contained 193 μL MLFS and 100 μL MILS.

Mixed amino-acids standard work solutions for signature peptide quantification: A MLFS contained 178 μL leucine stock solution, 100 μL proline stock solution, 207 μL valine stock solution and 9515 μL water. MILS contained 178 μL d_{10}-Leucine stock solution, 103 μL $^{13}C_5$-proline stock solution, $^{13}C_5$-valine stock solution and 9264 μL water. A lower level standard solutions contained 124 μL MLFS and 200 μL MILS. A higher level standard solution contained 193 μL MLFS and 200 μL MILS.
Peptide work solutions were prepared as followed with accurate weighing: A label free solution (LFS) contained 750 μL VR stock solution and 9250 μL water. An isotope labeled solution (ILS) contained 750 μL d_{10}-VR stock solution and 9250 μL water. A lower level standard solution contained 125 μL LFS and 200 μL ILS. A higher level standard solution contained 231 μL LFS and 200 μL ILS.

2. Supplementary Tables

Table 8 Uncertainty budget of signature peptide value assignment

	Sensitivity factor	Direct uncertainty	Uncertainty component
Weighing of leucine	2.47×10^{-5}	5.77×10^{-7}	1.43×10^{-11}
Weighing of water for leucine stock solution preparation	-2.45×10^{-8}	5.77×10^{-6}	-1.41×10^{-13}
Weighing of leucine stock solution	1.31×10^{-6}	5.77×10^{-6}	7.56×10^{-12}
The sample area ratio of leucine to leucine-d_{10}	4.25×10^{-4}	5.77×10^{-3}	2.45×10^{-6}
The standard area ratio of leucine to leucine-d_{10}	-3.98×10^{-4}	5.77×10^{-3}	-2.30×10^{-6}
Leucine purity	2.49×10^{-4}	4.62×10^{-3}	1.15×10^{-6}
The molecular weight of leucine	-1.89×10^{-6}	5.77×10^{-3}	-1.09×10^{-8}
The number of leucine in peptide	-8.27×10^{-5}	0.00	0
Weighing of proline	3.04×10^{-5}	5.77×10^{-7}	1.75×10^{-11}
Weighing of water for proline stock solution preparation	-3.14×10^{-8}	5.77×10^{-6}	-1.81×10^{-13}
Weighing of proline stock solution	3.03×10^{-6}	5.77×10^{-6}	1.75×10^{-11}
The sample area ratio of proline to proline-$^{13}C_5$	4.32×10^{-4}	5.77×10^{-3}	2.49×10^{-6}

The standard area ratio of proline to proline-$^{13}C_5$	-5.07×10^{-4}	5.77×10^{-3}	-2.92×10^{-6}
proline purity	3.13×10^{-4}	4.62×10^{-3}	1.45×10^{-6}
The molecular weight of proline	-2.71×10^{-6}	5.77×10^{-3}	-1.25×10^{-8}
The number of proline in peptide	-1.56×10^{-4}	0.00	0
Weighing of valine	2.41×10^{-5}	5.77×10^{-7}	1.39×10^{-11}
Weighing of water for valine stock solution preparation	-2.40×10^{-8}	5.77×10^{-6}	-1.38×10^{-13}
Weighing of valine stock solution	1.09×10^{-6}	5.77×10^{-6}	6.29×10^{-12}
The sample area ratio of valine to valine-$^{13}C_5$	4.59×10^{-4}	5.77×10^{-3}	2.65×10^{-10}
The standard area ratio of valine to valine-$^{13}C_5$	-4.28×10^{-4}	5.77×10^{-3}	-2.47×10^{-6}
Valine purity	2.45×10^{-4}	4.62×10^{-3}	1.13×10^{-6}
The molecular weight of valine	-2.08×10^{-6}	5.77×10^{-3}	-1.20×10^{-8}
The number of valine in peptide	-6.08×10^{-6}	0.00	0
Weighing of water for standard mixed amino acids	-8.02×10^{-8}	5.77×10^{-6}	-4.63×10^{-13}
Weighing of mixed standard amino acids	6.47×10^{-6}	5.77×10^{-6}	3.73×10^{-11}
Weighing of water for standard mixed label amino acids	-3.99×10^{-5}	5.77×10^{-6}	-2.30×10^{-10}
Weighing of the peptide sample solution	-8.04×10^{-5}	5.77×10^{-6}	-4.64×10^{-10}
Weighing of standard mixed label amino acids to peptide sample	5.20×10^{-6}	5.77×10^{-6}	3.00×10^{-11}
The molecular weight of peptide	4.29×10^{-7}	5.77×10^{-5}	2.48×10^{-11}
Weighing of peptide (solid)	-5.01×10^{-1}	5.77×10^{-7}	-2.89×10^{-7}
Weighing of water for peptide (solid)	5.11×10^{-4}	5.77×10^{-6}	2.95×10^{-9}

Table 9 Uncertainty budget of hCG value assignment (hCG enzymatic digestion)

	Sensitivity factor	Direct uncertainty	Uncertainty component
Weighing of peptide	4.74×10^{-4}	5.77×10^{-7}	2.73×10^{-10}
Weighing of water for peptide stock solution preparation	-4.79×10^{-8}	5.77×10^{-6}	-2.76×10^{-13}
Weighing of peptide stock solution	4.46×10^{-7}	5.77×10^{-6}	2.57×10^{-12}
The sample area ratio of peptide to peptide-(d_{10}-Leu)	10.00×10^{-5}	5.77×10^{-3}	5.77×10^{-7}
The standard area ratio of peptide to peptide-(d_{10}-Leu)	-1.48×10^{-4}	5.77×10^{-3}	-8.54×10^{-7}
Peptide purity	4.38×10^{-4}	4.62×10^{-3}	2.02×10^{-6}
The molecular weight of peptide	-1.87×10^{-7}	5.77×10^{-3}	-1.08×10^{-9}
The number of peptide in hCG	-3.60×10^{-4}	0.00	0
Weighing of water for standard mixed label peptides	-3.46×10^{-8}	5.77×10^{-6}	-2.00×10^{-13}
Weighing of mixed signature peptides	2.87×10^{-6}	5.77×10^{-6}	1.66×10^{-11}

Weighing of the hCG sample solution	-1.81×10^{-6}	5.77×10^{-6}	-1.04×10^{-11}
Weighing of standard mixed peptides to hCG sample	-9.28×10^{-6}	5.77×10^{-6}	-5.35×10^{-11}
Weighing of standard mixed label peptides to hCG sample	1.91×10^{-6}	5.77×10^{-6}	1.10×10^{-11}
The molecular weight of hCG	1.40×10^{-8}	5.77×10^{-5}	8.08×10^{-13}
Weighing of hCG (solid)	-5.01×10^{-1}	5.77×10^{-7}	-2.89×10^{-7}
Weighing of water for hCG (solid)	5.11×10^{-4}	5.77×10^{-6}	2.95×10^{-9}