Electronic Supplementary Material (ESI) for Analytical Methods.

A Novel Method Using ^{18}O and Metal Isobaric Labeling Combined with Multiple Reaction Monitoring Mass Spectrometry for the Absolute Quantification of a Target Proteome

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Preparation of a Concatemerof Q Peptides (QconCAT) of 22 Drug Metabolic Enzymes in Human Liver Microsomes.

The Construction of the QconCATP lasmid. The QconCAT plasmid was constructed by linking nucleotide fragments translated from 57 quantotypic peptides of 22 drug metabolic enzymes in sequence; this step was performed by Senggong Biotech Co. Ltd (Shanghai). According to the *E. coli* codon preferences, the nucleotide sequence was first optimized; the enzymatic digestion sites of BamH I and Xho I were inserted into the 5' and 3' ends of the gene; the plasmid and prokaryotic expression vector pGEX-4T-2 characterized by ampicillin resistance were simultaneously digested by BamH I and Xho I; and finally, the positive clones were screened and confirmed by sequencing the gene inserted in the plasmids by the Beijing Gene Institute (BGI, Beijing, China).

Transformation of the QconCAT Plasmid. A total of 100 μL of competent *Escherichia coli* cells BL21 (DE3) were thawed on ice for 30 min. After gently mixing with 1 μL of plasmids, the mixture was incubated on ice for another 30 min. The bacterial cells were heat-shocked in a water bath at 42 ℃ for 90 seconds and then put immediately into an ice bath for 2 min. A total of 0.4 mL of LB medium was added before the bacterial cells were incubated on a constant temperature shaker at 150 rpm/min at 37℃for 1 h. The bacterial solution was centrifuged at 5000 rpm/min for 3 min, then resuspended after 200 μL of supernatant was removed before it was coated uniformly on the surface of an agar plate containing ampicillin. The plate was allowed to stand at 37℃ for 1 h for suspension fluid evaporation, then inverted and cultured overnight.

Expression of the QconCAT Protein. Positive clones were picked and inoculated in 10 mL of selective LB liquid medium before they were incubated on a shaker at 180 rpm/min at 37℃ overnight. The next day, 15 μL of the bacterial solution was inoculated in 5 mL LB liquid medium containing ampicillin, and the bacterial solution was incubated on a shaker at 200 rpm/min at 37℃for approximately 2.5 h until the optical density (OD 600) reached 0.6 to 0.8. Then, 20 μL of the bacterial solution was centrifuged at 12000 g for 1 min, the supernatant was discarded and the pellet was stored at -20℃ as a control sample before induction expression. A total of 0.5 mol/L IPTG was added to the residual bacterial solution at a final concentration of 0.5 mmol/L, and the bacterial solution was cultured on a shaker at 200 rpm/min at 37℃ for 2 h. A total of 20 μL bacterial solution was collected after induction expression and stored at 4℃ as a sample. A total of

2×SDS loading buffer was separately added to the sample and the control sample and both samples were boiled for 5 min followed by SDS-PAGE. The gel was stained with Coomassie Brilliant Blue R250 for 1 h and then destained until the protein bands were clearly visible. According to the experimental results, successfully expressed *E. coli* clones were first selected for large-scale induction expression, then collected and stored at -20℃ for purification and analysis in the next experiment.

Lysis of the Expressed *E. coli* Cells. A total of 25 mL of the bacterial pellet was added to 800 μL of PBS for resuspension; then 16 μL of protease inhibitor was added, and finally the cells were broken by ultrasonication (power ＜ 400 W, ultrasonicated for 10 seconds and then stopped for 10 seconds, 90 cycles in total) until the bacterial solution was clear.

Purification of the QconCAT Protein. A total of 80 μL of glutathione sepharose packing materials was added to a 1.5 mL Eppendorf tube, and the Eppendorf tube was centrifuged at 500 g for 5 min, and then the supernatant was discarded. Another 500 μL of PBS (pH 7.3) was added to the Eppendorf tube to equilibrate the packing materials, after which it was centrifuged at 500 g for 5 min, the supernatant was removed again, and the above process was performed twice. The bacterial lysate was added to the Eppendorf tube containing packing materials and incubated at 4℃ for 1 hour. The Eppendorf tube was then centrifuged at 4℃ for 5 min, 10 μL of the supernatant was collected for SDS-PAGE analysis, and the remainder was discarded. The precipitate was added to 500 μL of PBS buffer (pH 7.3) before centrifuging at 500 g for 5 min. The supernatant was removed, and elution buffer (50 mmol/L Tris/HCl, 10 mmoL/L reduced glutathione) was added to half the volume of the packing materials. After mixing and centrifuging at 500 g for 5 min, the supernatant was collected, and the elution process was performed once again. These two supernatants were pooled together and stored at -80℃ for use in the following experiments.

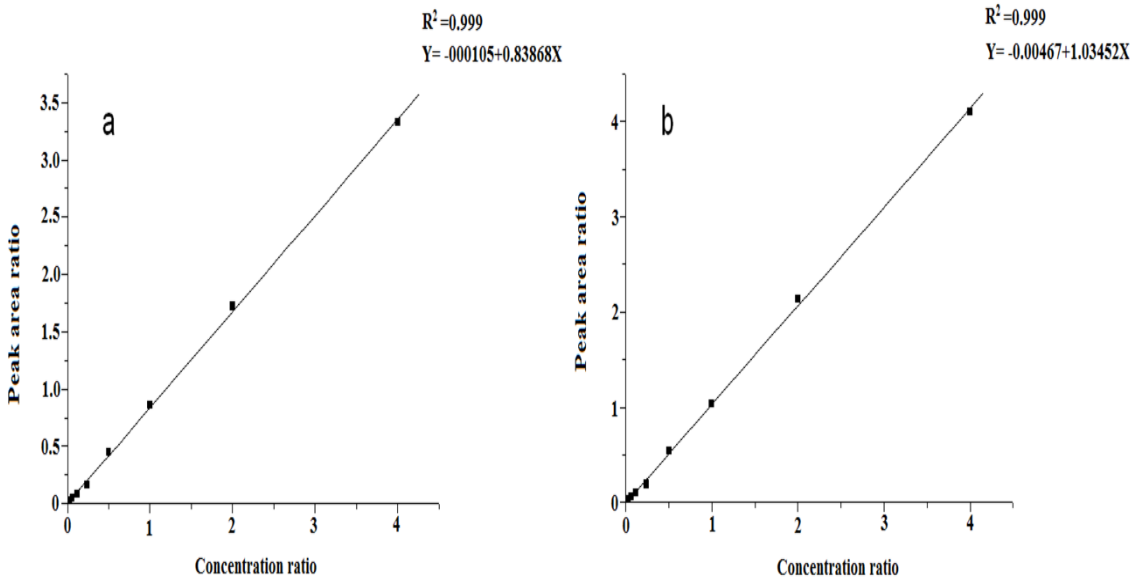

Figure S1 Calibration curves of the transitions of an ^{18}O/metal labeled peptide (TATVDDIDNIYR)

a, the linear curve of the transition of a y5 ion; b, the linear curve of the transition of a b2 ion

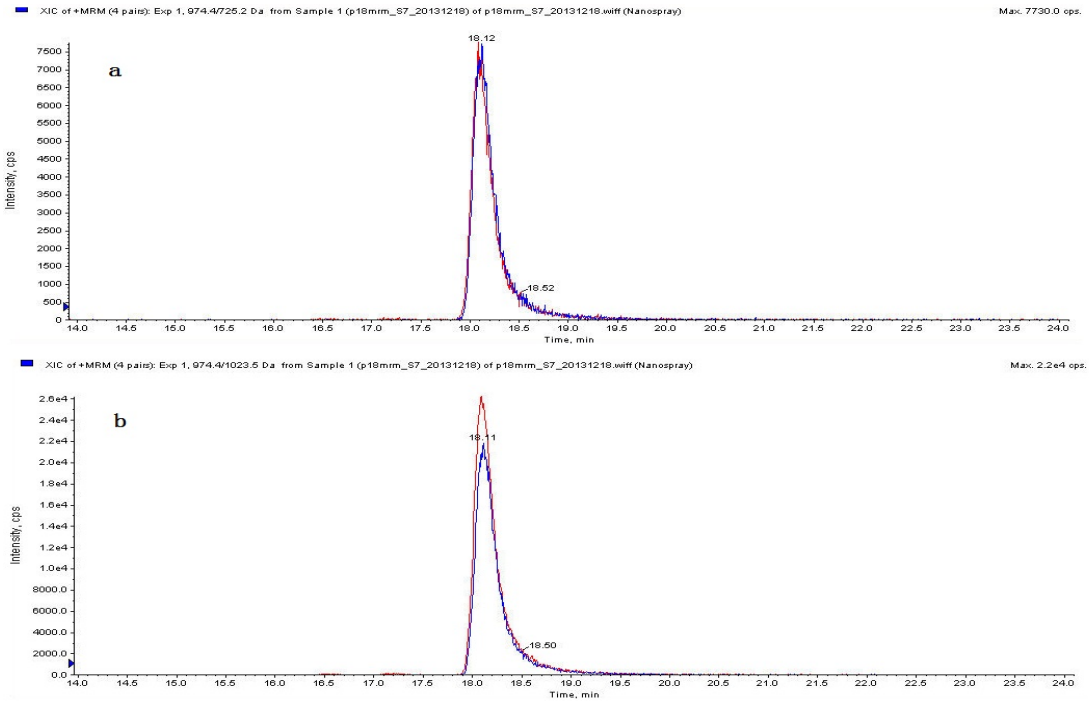

Figure S2 TICs of the two transitions of ^{18}O+Ho or ^{16}O+Tm labeled peptides

a, TICs of transitions: 974.5/1023.5 and 974.5/1027.5 (y5 ion); b, TICs of transitions: 974.5/725.2 and 974.5/721.2 (b2 ion)

Table S1 The transitions, their CE and DP values of a labeled peptide (TATVDDIDNIYR) when performing MRM MS analysis

Signature Peptide	Parent ion	Produce ion	CE	DP
Tm-DOTA-TATVDDIDNIYR	974.5	1023.5（y5）	50	120
	974.5	725.2（b2）	66	120
Ho-DOTA-TATVDDIDNIYR-^{18}O	974.5	1027.5（y5）	50	120
	974.5	721.2（b2）	66	120

Table S2 The relative errors of seven standard samples calculated by the calibration curves

Standard samples	Tm-P （fmol/ul）	Ho-P-^{18}O （fmol/ul）	Relative errors (RE%)	
			y ions	b ions
1	0.7	75	14.21	18.04
2	1.5	75	0.61	4.79
3	3	75	12.68	0.36
4	6	75	8.77	3.64
5	12	75	0.39	0.17
6	50	75	4.06	1.64
7	100	75	0.97	0.39

Table S3 Equations of calibration curves and the corresponding linear correlation coefficients

Protein	Peptide	Transitions of ^{16}O+Tm/ ^{18}O+Ho labeled peptides	Linear equations	R^2
CYP 1A2	IGSTPVLVLSR	783.5/787.5	y=0.9929x-0.0062	0.99
		375.2/379.2	y=1.09503x-0.0127	0.99
	ASGNLIPQEK	995.4/991.4	y=0.97741x+0.02221	0.99
		543.3/547.3	y=1.01484x+0.03074	0.99
	GYGVVFSNGER	929.3/925.3	y=1.018x+0.02346	0.99
		808.4/812.4	y=0.40198x+0.10928	0.98
CYP 2A6	GTGGANIDPTFFLSR	1123.4/1119.4	y=0.67783x-0.1361	0.99
		867.5/871.5	y=0.82117x-0.14743	0.99
	GTEVYPMLGSVLR	840.2/836.2	y=0.67465x-0.03517	0.98
		531.3/535.3	y=0.87861x-0.04541	0.99
CYP 2B6	IAMVDPFFR	681.3/685.3	y=0.74844x+0.11335	0.99
		566.3/570.3	y=0.74676x+0.04052	0.99
	DLIDTYLLHMEK	781.2/777.2	y=0.86394x-0.1253	0.98
		586.3/590.3	y=0.84209x-0.08596	0.98
CYP 2C8	DQNFLTLMK	534.3/538.3	y=0.99701x-0.04693	0.99
		433.3/437.3	y=0.98335x-0.00475	0.99
	VQEEIDHVIGR	581.4/585.4	y=1.31772x+0.02538	0.99
		696.4/700.4	y=1.07941x-0.00243	0.99
CYP 2C9	GIFPLAER	585.3/589.3	y=1.15702x-0.00864	0.99
		375.2/379.2	y=1.09969x+0.012	0.99
	SHMPYTDAVVHEVQR	777.2/773.2	y=1.16554x-0.02041	0.99
		668.3/672.3	y=1.38453x+0.01498	0.99
CYP 2C19	NLAFMESDILEK	780.2/776.2	y=0.93109x-0.00521	0.99
		851.3/847.3	y=0.65221x+0.11177	0.98
CYP 2E1	GDLPAFHAHR	725.1/721.1	y=0.84196x+0.01352	0.99
		520.3/524.3	y=0.5955x+0.09915	0.98
	EAHFLLEALR	1150.4/1146.4	y=1.13419x-0.05365	0.99
		861.5/865.5	y=1.27543x-0.02417	0.99
CYP 2D6	FITLVPSNLPHEATR	813.3/809.3	y=0.5183x+0.05659	0.98
		710.4/714.4	y=0.8961x-0.05326	0.99
	DIEVQGFR	507.3/511.3	y=1.02349x+0.01862	0.99
		379.2/383.2	y=0.91425x+0.01001	0.99
CYP 3A4	SAISIAEDEEWK	824.3/820.3	y=1.50788x-0.10543	0.99
		948.4/952.4	y=1.36644x-0.09581	0.98
	GVVVMIPSYALHR	709.3/705.3	y=0.87289x-0.02574	0.99
		843.4/847.1	y=1.01296x-0.08387	0.99
CYP 3A5	DTINFLSK	536.1/540.1	y=0.52794+0.20447	0.99

		650.4/654.4	y=0.62159x+0.16015	0.99
UGT 1-1	DGAFYTLK	566.3/570.3	y=0.77029x+0.08908	0.99
		403.3/407.3	y=1.03658x+0.00185	0.99
	VLVVPTDGSPWLSMR	864.3/860.3	y=0.966811x-0.05236	0.99
UGT 1-4	YIPCDLDFK	829.0/825.0	y=0.91546x-0.09439	0.98
		679.3/683.3	y=0.72316x-0.04095	0.99
	GTQCPNPSSYIPK	999.2/995.2	y=0.76797x+0.00992	0.99
		833.5/837.5	y=0.58496x+0.08738	0.99
	DIVEVLSDR	589.3/593.3	y=1.02349x+0.01862	0.99
		718.4/722.4	y=0.91425x+0.01001	0.99
UGT 1-6	SFLTAPQTEYR	793.4/797.4	y=1.03214x+0.00257	0.99
		864.4/868.4	y=0.60239x-0.00684	0.99
	DVDIITLYQK	694.4/698.4	y=0.78668x-0.0358	0.99
		480.3/484.3	y=0.98815x-0.06592	0.99
UGT 1-9	AFAHAQWK	771.0/767.0	y=1.19315x+0.00849	0.99
		574.3/570.3	y=1.010152+0.03741	0.99
	TILDELVQR	759.4/763.4	y=0.54971x-0.05449	0.99
		644.4/648.4	y=0.77577x-0.07312	0.99
UGT 2B4	FSPGYAIEK	502.3/506.3	y=1.04419x-0.01606	0.99
		431.3/435.3	y=1.01708x+0.013	0.99
	ANVIASALAK	950.3/946.3	y=1.02381x+0.01261	0.99
		837.2/833.2	y=1.13796x-0.03255	0.99
UGT 2B10	HSGGFIFPPSYVPVVMSK	702.4/706.4	y=0.67787x+0.05974	0.99
		623.3/625.3	y=0.58737x-0.05522	0.98
UGT 2B11	FEVYPTSLTK	829.2/825.2	y=0.72094x-0.01496	0.99
		851.5/855.5	y=1.04567x-0.03582	0.99
	FSVGYTFEK	786.4/790.4	y=0.97678x-0.03697	0.99
		566.3/570.3	y=0.73099-0.01692	0.99
UGT 2B15	SVINDPVYK	548.3/552.3	y=1.00756x+0.01856	0.99
		777.4/781.4	y=1.01384x+0.03181	0.99
	FAVFGLGNK	530.3/534.3	y=0.99279x-0.03236	0.99
		677.4/681.4	y=1.12068x-0.00666	0.99
POR	GVATNWLR	588.3/592.3	y=0.66051x+0.20278	0.99
		689.4/693.4	y=0.78434x+0.17301	0.99
	YYTLEEIQK	688.4/692.4	y=0.95811x-0.03602	0.99
		559.3/563.3	y=1.00752x-0.0536	0.99
Cytb5	STWLILHHK	927.2/923.2	y=1.69963x+0.33901	0.98
		463.3/467.3	y=2.27977x+0.16949	0.99
	FLEEHPGGEEVLR	1208.4/1204.4	y=1.05749x-0.0244	0.99
		856.5/860.5	y=0.95941x-0.01807	0.99

Table S4 Comparison with reported data

Drug metabolizing enzymes	This paper Mean (fmol/μg)	Reference [35] Range (fmol/μg)	Reference [36] Range (fmol/μg)
CYP1A2	75.3	2.9 – 103.9	3.26-65.5
CYP2A6	89.3	13.6 – 190.8	5.45-168
CYP2B6	21.3	1.1 – 173.7	4.05-14.9
CYP2C8	83.4		5.66-83.5
CYP2C9	130.9	4.4 – 79.4	40.2-115
CYP 2C19	121.3		2.02-22.2
CYP2D6	16.8	0.1 − 62.4	6.16-36.4
CYP3A4	67.5	10.4 − 262.1	6.22-270
CYP3A5	65.5	0.6 − 57.2	2.48-17.1
CYP2E1	72.5		36.3-147
UGT1A1	28.6	8.9 − 137.9	20.8-59.7
UGT1A4	101.3	14.4 − 105.6	
UGT1A6	21.6	31.5 − 285.4	45.0-277
UGT1A9	18.5	13.4 − 122.6	15.5-38.0
UGT2B4	111.8	22.8 − 135.8	
UGT2B10	204.6		
UGT 2B11	10.2		
UGT2B15	75.4	18.4 − 130.2	24.2-103
POR	61.9		41.7-99
Cytb5	465.02		