

Supporting Information(SI)

**A New Sample Preparation Method for the Absolute Quantitation of a
Target Proteome using ^{18}O Labeling Combined with Multiple Reaction**

Monitoring Mass Spectrometry

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Table S1 57 peptides chosen from 22 drug-metabolizing enzymes

No.	Peptide sequence	GI number	protein name ^a
1	IGSTPVLVLSR	73915100	CYP 1A2
2	FLWFLQK	73915100	CYP 1A2
3	ASGNLIPQEK	73915100	CYP 1A2
4	GYGVVFSNGER	189339233	CYP 2A6
5	GTGGANIDPTFFLSR	189339233	CYP 2A6
6	GTEVYPMLGSVLR	189339233	CYP 2A6
7	IAMVDPFFR	7949031	CYP 2B6
8	DLIDTYLLHMEK	7949031	CYP 2B6
9	EALIDNGEEFSGR	98991773	CYP 2C8
10	DQNFLTLMK	98991773	CYP 2C8
11	VQEEIDHVIGR	98991773	CYP 2C8
12	GIFPLAER	13699818	CYP 2C9
13	SHMPYTDAVVHEVQR	13699818	CYP 2C9
14	GTILISLTSVLHDNK	13699818	CYP 2C9
15	NLAFMESDILEK	4503219	CYP 2C19
16	SNYFMPFSAGK	4503219	CYP 2C19
17	GDLPAFHAHR	10834998	CYP 2E1
18	EAHFLEALR	10834998	CYP 2E1
19	FITLVPSNLPHEATR	10834998	CYP 2E1
20	AFLTQLDELLTEHR	392513721	CYP 2D6
21	DLTEAFLAEMEK	392513721	CYP 2D6
22	DIEVQGFR	392513721	CYP 2D6
23	SAISIAEDEEWK	13435386	CYP 3A4
24	GVVVMIPSYALHR	13435386	CYP 3A4
25	SAISLAEDEEWK	4503231	CYP 3A5
26	DTINFLSK	4503231	CYP 3A5
27	GHEIVVLAPDASLYIR	8850236	UGT 1A1
28	DGAFYTLK	8850236	UGT 1A1
29	VLVVPIDGSHWLSMR	13487900	UGT 1A3
30	YLSIPTVFFLR	13487900	UGT 1A3
31	VLVVPTDGSPWLSMR	6005930	UGT 1A4
32	YIPCDLDFK	6005930	UGT 1A4
33	GTQCPNPSSYIPK	6005930	UGT 1A4
34	DIVEVLSDR	45827765	UGT 1A6
35	SFLTAPQTEYR	45827765	UGT 1A6
36	DVDIITLYQK	45827765	UGT 1A6
37	AFAHAQWK	11276085	UGT 1A9
38	NHIMHLEEHLCHR	11276085	UGT 1A9
39	TILDELVQR	149944509	UGT 2B4
40	FSPGYAIEK	149944509	UGT 2B4
41	ANVIASALAK	149944509	UGT 2B4
42	TILDELIQR	190194389	UGT 2B7

43	IEIYPTSLTK	190194389	UGT 2B7
44	ANVIASALAQIPQK	190194389	UGT 2B7
45	TEFENIMQLVK	4507817	UGT 2B10
46	HSGGFIFPPSYVPVMSK	4507817	UGT 2B10
47	FEVYPTSLTK	4507823	UGT 2B11
48	AVFWIEFVMPHK	4507823	UGT 2B11
49	NYLEDSLLK	116517299	UGT 2B15
50	FSVGYTFEK	116517299	UGT 2B15
51	SVINDPVYK	116517299	UGT 2B15
52	FAVFGLGNK	127139033	POR
53	GVATNWLR	127139033	POR
54	DGALTQLNVAFSR	127139033	POR
55	YTTLEEIQK	41281768	Cyt b5
56	STWLILHHK	41281768	Cyt b5
57	FLEEHPGGEEVLR	41281768	Cyt b5

a, CYP, cytochrome P450; UGT, uridine 5'-diphosphoglucuronosyltransferase; Cyt: Cytochrome.

As shown in Figure S1, the number of peptides with one missed cleavage site after 16 h of digestion was selected to examine the digestion efficiency at different trypsin to protein ratios. For example, when fewer than 15 peptides with one missed cleavage site was chosen as the criterion, we found that the digestion times lasted more than 16 h, 10 h, 8 h, 6 h or 45 min when the digestion was performed at trypsin to protein ratios of 1:50, 1:25, 1:10, 1:5 and 1:1. The results indicated that the numbers of peptides with one missed cleavage site decreased as the trypsin to protein ratios increased.

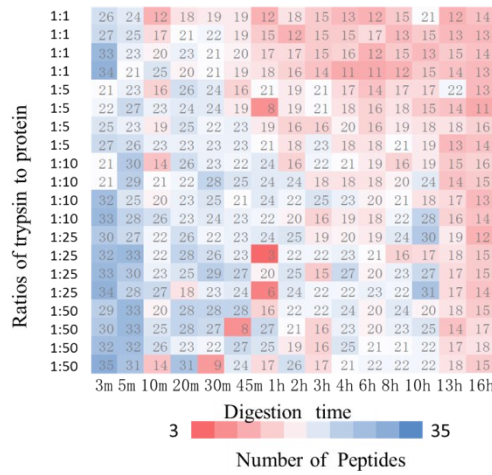


Fig. S1 The numbers of peptides with one missed cleavage sites varies with time at different trypsin to protein ratios

Similarly, as shown in Figure S2, when, averagely, 7 peptides with two missed cleavage sites identified after 16 h digestion at different ratios of trypsin to protein is selected to examine the digestion efficiency, we found that digestion times lasted more than 16 h, 16 h, 13 h, 6 h or 1 h when the digestion at trypsin to protein ratios of 1:50, 1:25, 1:10, 1:5 and 1:1 was performed. These results further suggest that high trypsin concentration benefits complete digestion.

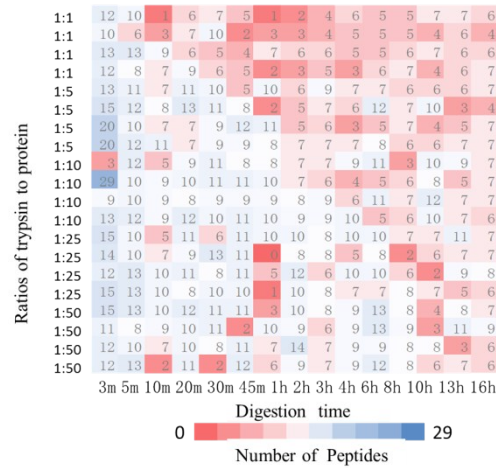


Fig. S2 The numbers of peptides with two missed cleavage sites varies with time at different trypsin to protein ratios

As shown in Figure S3, when we chose 53 peptides with no more than two missed cleavage sites after 16 h of digestion at different ratios of trypsin to protein, at least 16 h, 16 h, 13 h, 2 h or 1 h were needed for digestion at trypsin to protein ratios of 1:50, 1:25, 1:10, 1:5 and 1:1, respectively. These results suggest that the numbers of peptides with no more than two missed cleavage sites decrease sharply when the trypsin to protein ratios increased. All statistical results suggest that a high ratio of trypsin to protein benefits complete digestion.

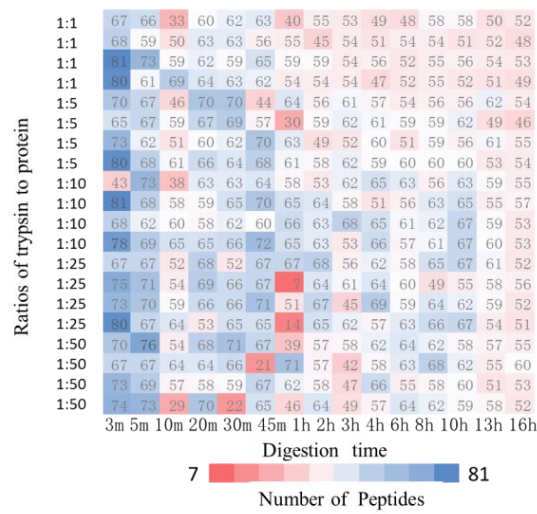


Fig. S3 The numbers of peptides with two or fewer missed cleavage sites varies with time at different trypsin to protein ratios

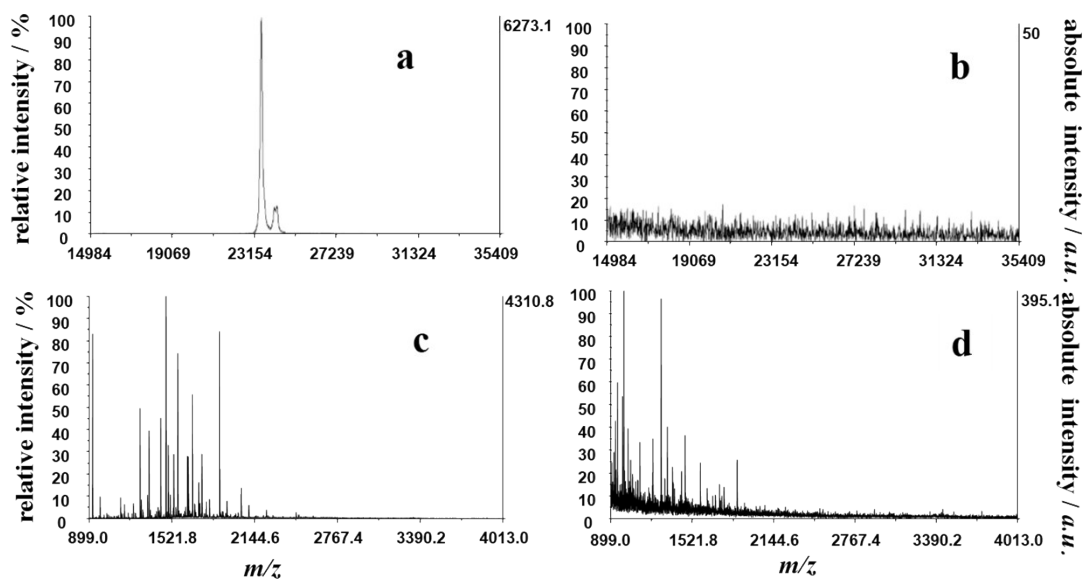


Fig. S4 MALDI-TOF MS spectra of the original trypsin solution, BSA tryptic digest and their flowthrough fractions after loading samples on a reversed-phase pipette tip prepared in house. (a) original trypsin solution; (b) flowthrough fraction after loading trypsin on the reversed-phase pipette tip; (c) original BSA digest; (d) flowthrough fraction after loading BSA digest on the reversed-phase pipette tip

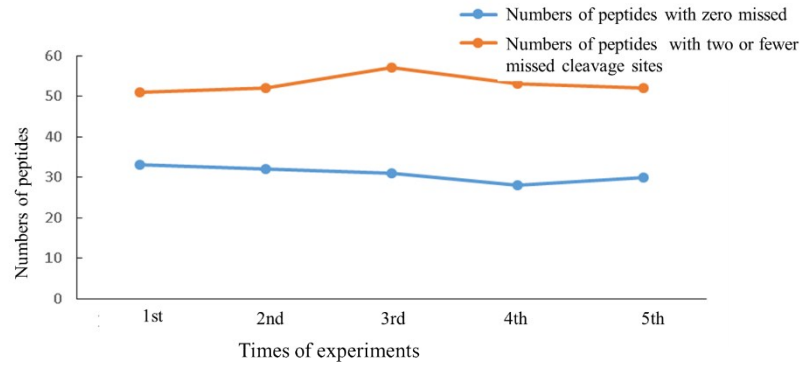


Fig. S5 Numbers of peptides with zero missed cleavage sites or with two or fewer missed cleavage sites analyzed via MALDI-TOF MS of the BSA digest using trypsin recycled five times

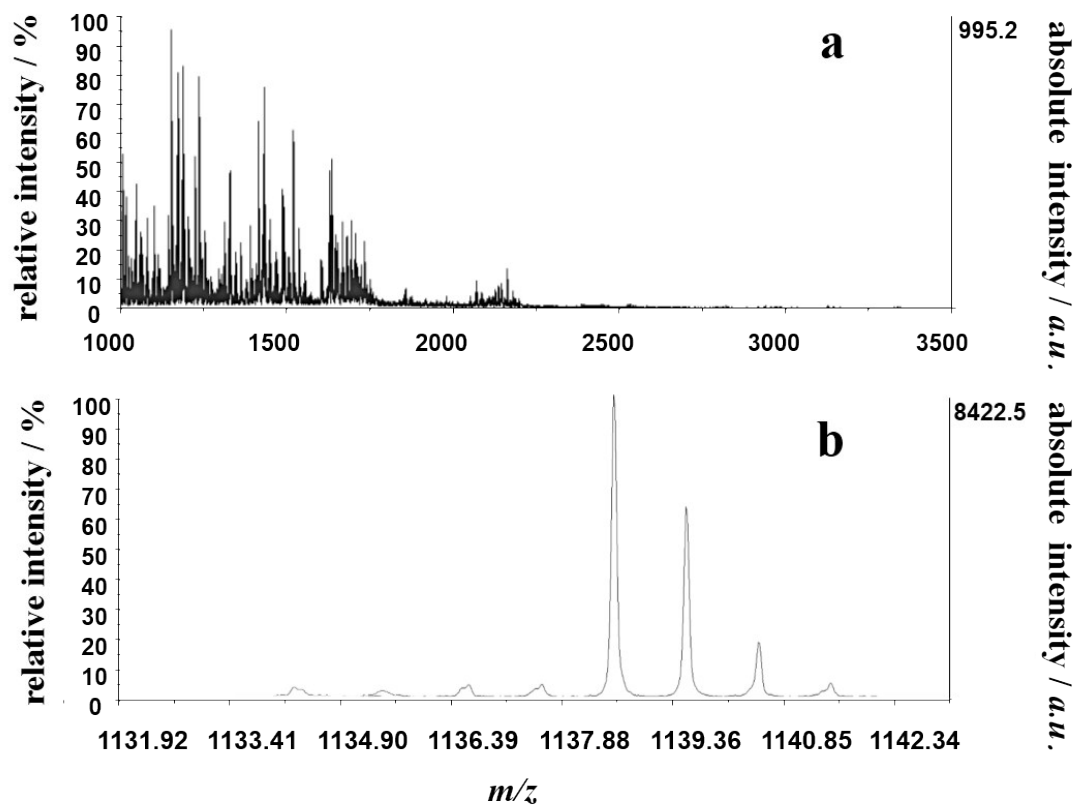


Fig. S6 MALDI-TOF MS spectra of (a) a QconCAT digest and (b) one peptide YIPCDLDFK (m/z 1134.4 unlabeled with ^{18}O , m/z 1138.5 labeled with Two ^{18}O) of the QconCAT selected randomly

Table S2. MRM parameters and analytical precision of MRM assays based on tryptic peptides from 22 drug- metabolizing enzymes

Proteins	Peptide sequences	Q1 ^a (m/z)	Q3 ^b (m/z)	Linear equations	R ²	Quantitation	Average	RSD(%)
CYP 1A2	IGSTPVLVLSR	571.4	783.5	y=36213.39x-894238.82	0.99	182.05	163.18	17.13%
			1028.6	y=7560.79x-182590.45	0.99	177.95		
			587.4	y=5637.34x-118878	0.99	214.97		
	FLWFLQK	491.3	721.4	y=1481.11x-37911.32	0.97	127.00		
			388.3	y=260.81x-3614.61	0.96	136.18		
			501.3	y=20246.78x+116796.08	0.99	157.91		
ASGNLIPQEK	528.8	614.4	y=7107.12x+57940.17	0.99	153.48			
		898.5	y=1761.3x+17317.26	1	155.93			
		709.3	y=1549.9x-30733.6	0.98	43.54			
CYP 2A6	GYGVVFSNGER	592.8	808.4	y=2066.6x-58627.48	0.97	42.00	40.47	17.19%
			562.3	y=532.26x-5816.91	0.95	44.81		
			867.5	y=20895.11x-912973.11	0.95	45.70		
	GTGGANIDPTFFLSR	776.9	982.5	y=14309.68x-642552.76	0.97	37.77		
			872.5	y=9390.25x-393417.13	0.99	25.81		
			1035.6	y=3221.06x-122834.61	0.99	43.64		
GTEVYPMLGSLVLR	711.4	780.4	y=4053.21x-175543.01	0.98	20.18			
		566.3	y=20521.13x-857118.12	0.99	15.44			
		933.5	y=2807.58x-152636.45	0.95	23.95			
CYP 2E6	IAMVDPFFR	548.3	657.3	y=2907.78x-129241.8	0.96	20.51	19.82	15.46%
			770.4	y=3085.86x-128558.64	0.96	18.99		
	DLIDTYLLHMEK	745.9	866.5	y=4470.27x-75806.97	0.99	17.24		
			492.3	y=2610.03x-46087.89	0.99	18.69		
CYP 2C8	DQNFLTLMK	555.3	605.4	y=2876.86x-70151.95	0.99	20.94	17.97	5.72%
			668.3	y=3537.63x-139281.97	0.98	66.10		
			767.4	y=3146.49x-134857.46	0.98	66.97		
CYP 2C9	SHMPYTDAVVHEVQR	590.3	937.5	y=1436.49x-61066.57	0.98	67.73	66.93	1.22%
			668.3	y=3537.63x-139281.97	0.98	66.10		
			767.4	y=3146.49x-134857.46	0.98	66.97		
CYP 2C19	NLAFMESDILEK	705.4	1111.5	y=2010.76x-53287.3	0.99	26.62	28.80	7.75%
			968.5	y=1101.8x-35023.2	0.99	31.08		
			1186.6	y=1032.29x-40620.66	0.99	28.71		
CYP 2E1	GDLPAFHHR	560.8	520.3	y=4146.32x-31569.78	0.99	42.73	59.55	20.07%
			835.4	y=9098.51x-98542.81	0.98	42.98		
			861.5	y=6851.46x-312242.19	0.96	68.77		
	EAHFLLEALR	599.8	714.5	y=6051.51x-266057.11	0.96	68.44		
			601.4	y=3129.77x-130049.51	0.96	68.76		
			1121.6	y=22181.19x-870989.88	0.98	58.56		
CYP 2D6	DLTEAFLAEMEK	848.0	1220.6	y=446.25x-7175.61	0.98	66.62	37.25	5.09%
			867.4	y=3721.56x-167883.07	0.96	34.86		
			607.3	y=2482.48x-113295.02	0.95	35.45		
CYP 3A4	GVVVMIPSYALHR	482.2	720.4	y=2621.99x-121476.18	0.95	36.56	38.65	
			735.4	y=12105.88x-68147.44	0.99	39.53		
			606.3	y=5994.92x-46870.87	0.99	38.65		
			507.3	y=3588.53x-17136.35	1	38.42		
CYP 3A4	GVVVMIPSYALHR	721.4	843.4	y=3758.35x-117995.06	0.98	23.42	22.35	
			1087.6	y=853.58x-14834.98	0.97	22.35		

CYP 3A5	DTINFLSK	469.3	835.3	$y=2546.12x-51193.03$	0.96	21.19	23.98	10.57%	
			721.4	$y=9113.2x-314735.97$	0.96	26.14			
			608.3	$y=6376.8x-203124.87$	0.96	24.62			
UGT 1A1	DGAFYTLK	457.7	524.3	$y=2472.93x+35563.45$	0.97	8.06	9.78	18.21%	
			361.2	$y=1069.46x+22267.83$	0.98	9.67			
			973.5	$y=260.97x+2292.57$	0.96	130.66			
UGT 1A3	VLVVPIDGSHWLSMR	855.0	1298.6	$y=458.17x-6730.44$	0.98	101.85	116.25	17.52%	
			1246.6	$y=4303.75x-109154.39$	0.97	58.10			
UGT 1A4	VLVVPTDGSPWLSMR	828.9	933.5	$y=1993.43x+74264.09$	0.98	48.34	59.08	9.67%	
			789.4	$y=769.98x-13470.74$	0.98	64.97			
			585.8	894.4	$y=15137.93x-354295.6$	0.99			62.13
			YIPCDLDFK	637.3	$y=1119.05x-6284.54$	0.99			61.18
				797.3	$y=1819.95x-13873.92$	0.99			61.15
UGT 1A6	DIVEVLSDR	523.3	724.8	$y=12271.07x-401305.47$	0.98	53.77	26.36	15.51%	
			791.4	$y=3556.94x-143621.51$	0.97	55.71			
			905.5	$y=670.86x-13891.83$	0.99	66.34			
			817.4	$y=11995.76x-185284.47$	1	23.51			
			718.4	$y=12442.71x-192096.99$	1	23.89			
UGT 1A6	SFLTAPQTEYR	656.8	589.3	$y=8982.21x-120005.11$	1	24.26	26.36	15.51%	
			793.4	$y=21878.21x-548043.85$	0.99	20.46			
			965.5	$y=10772.73x-263090.31$	0.99	25.66			
			864.4	$y=6964.74x-150174.16$	1	25.27			
			993.6	$y=3344.31x-103090.14$	0.96	30.46			
UGT 1A9	AFAHAQWK	479.7	604.3	$y=4519.47x-132929.62$	0.96	31.10	54.66	3.86%	
			765.5	$y=2636.42x-80497.7$	0.96	32.63			
			652.4	$y=3476.25x-69086.8$	1	56.88			
			532.3	$y=5354.59x-142128.68$	1	52.69			
			740.4	$y=3419.7x-101763.25$	1	54.41			
UGT 2B4	TILDELVQR	543.8	872.5	$y=35442.4x-1358536.28$	0.98	73.65	83.73	11.65%	
			644.4	$y=8689.22x-338672.57$	0.95	72.34			
			759.4	$y=13289.37x-428197.1$	0.98	72.55			
			777.4	$y=10345.45x-337.08$	1	86.77			
			680.4	$y=4118.63x+2350.65$	0.99	86.79			
UGT 2B7	IEIYPTSLTK	582.8	506.3	$y=942.44x+6177.41$	0.98	82.49	97.09	5.31%	
			460.3	$y=9809.69x-65579.43$	0.98	91.03			
			673.4	$y=7059.07x+12384.54$	0.97	101.79			
			560.3	$y=4099.81x-27407.55$	0.98	86.13			
			772.5	$y=11837.97x-121520.76$	0.99	103.05			
UGT 2B10	TEFENIIMQLVK	732.9	922.5	$y=9958.01x-214468.42$	0.99	93.99	180.03	3.65%	
			809.4	$y=7943.5x-144344.39$	0.99	94.23			
			958.6	$y=692.49x-4394.9$	0.96	184.68			
UGT 2B11	FEVYPTSLTK	592.8	844.5	$y=363.11x+7281.94$	0.96	175.39	86.91	3.82%	
			809.4	$y=8365.42x-172273.42$	0.99	84.56			
UGT 2B15	NYLEDLLK	547.8	809.4	$y=13160.59x-209073.57$	0.99	89.25	65.73	11.62%	
			704.4	$y=5265.94x-101042.66$	1	67.32			
			575.3	$y=1761.2x-33793.4$	0.99	61.22			
UGT 2B15	SVINDPVYK	517.8	460.3	$y=1200.97x-5628.95$	0.99	53.15	69.41	11.62%	
			848.5	$y=11635.85x-115621.45$	0.99	69.41			
			506.3	$y=5486.76x-12276.67$	0.99	75.33			

POR	GVATNWLR		735.4	$y=5765.06x-15953.22$	0.99	67.96	103.50	10.69%
			760.4	$y=5444.56x-201719.83$	0.95	105.67		
			588.3	$y=1205.95x-38136.62$	0.96	118.52		
			409.2	$y=430.84x+28734.48$	0.96	93.47		
			806.5	$y=858.83x-11174.18$	0.99	91.78		
Cyt b5	YITLEEIQK	593.8	696.4	$y=586.4x-155.53$	0.99	108.08	576.71	5.12%
			860.5	$y=13201.99x-224731.1$	0.98	546.30		
			646.3	$y=3171.29x-52545.23$	0.98	605.26		
			759.4	$y=3522.65x-58297.85$	0.99	578.56		

^a Endogenous peptide precursor m/z ; ^b Fragment ion.