

Figure S1. The CoA biosynthetic pathway with corresponding structures: As in Figure 1, the metabolites detected by the method detailed in this manuscript are highlighted in green. PANK, pantothenate kinase; PPCS, phosphopantothenoylcysteine synthase; PPCDC, phosphopantothenoylcysteine decarboxylase; PPAT, phosphopantetheine adenylyl transferase; DPCK, dephosphocoenzyme A kinase. The bifunctional enzyme Coenzyme A synthase (COASY) is comprised of PPAT and DPCK.

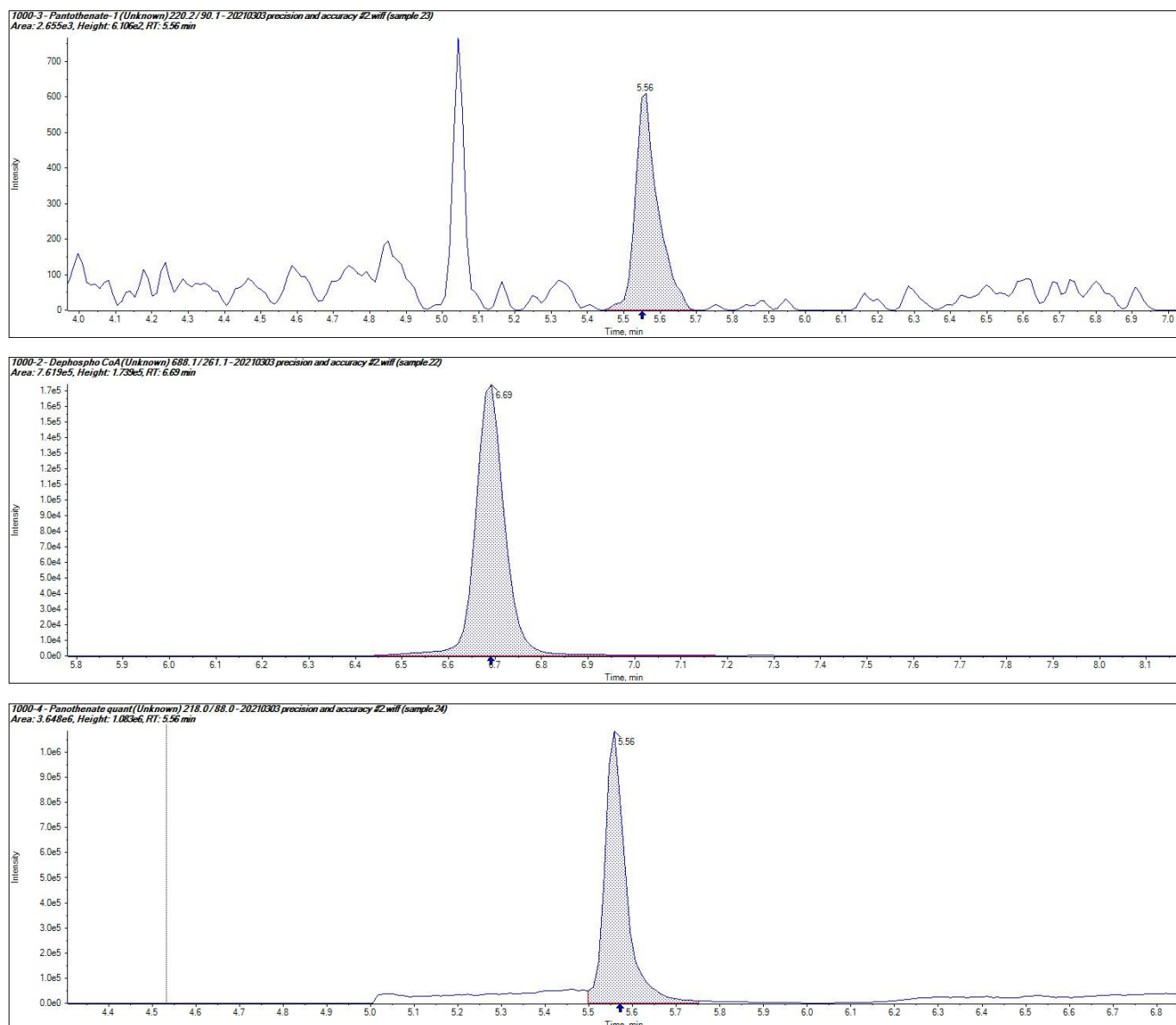
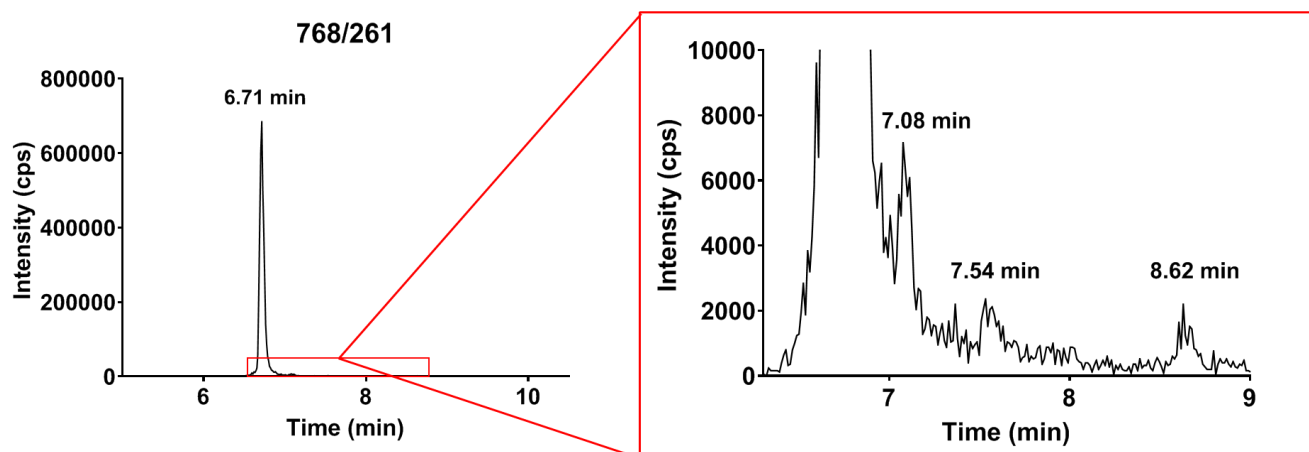


Figure S2. Pantothenate is only detectable with adequate sensitivity with negative-mode MRM: (*Top*) Pantothenate analyzed with positive-mode MRM. Even with optimized MRM settings, the signal is barely above the lower limit of detection (5X the noise floor). (*Middle*) Dephospho-CoA detected with positive-mode MRM with 200-fold more intensity than pantothenate. (*Bottom*) Pantothenate detected with negative-mode MRM with 1×10^3 the signal intensity of positive-mode. All analytes were measured at 5 pmol/ μ L.



Combined transitions

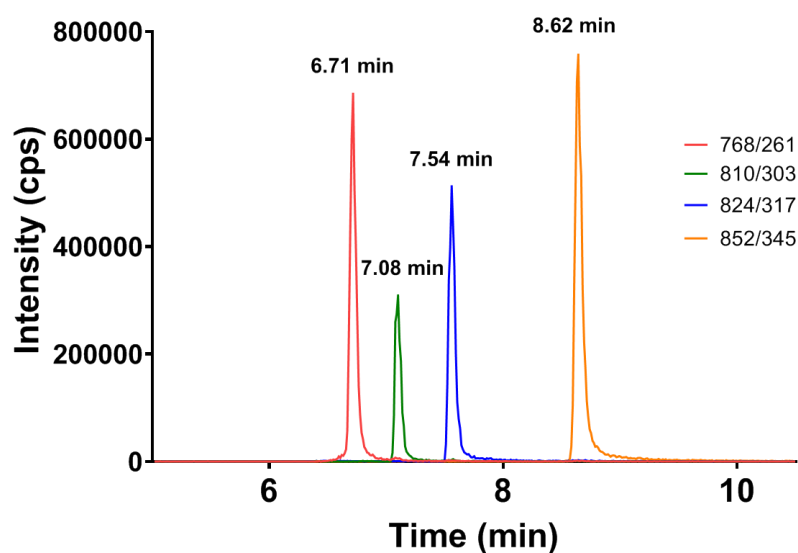


Figure S3. Free CoA is produced in the ESI source from acetyl, propionyl, and isovaleryl CoA: Standards were injected and the MRM channel associated with CoA (768/261) was monitored. Peaks of 768/261 were observed at 6.71 min, the retention time of CoA-SH (*top panel, left*); as well as at 7.08, 7.54, and 8.62 (*top panel, inset*). The retention times of 7.08, 7.54, and 8.62 were otherwise associated with dominant peaks for acetyl CoA (810/303), propionyl CoA (824/317), and isovaleryl CoA (852/345) (*bottom panel*).

Table S2. Determination of matrix effect

	Stds spiked in	Stds in post-extraction matrix (AUC)	Stds in extraction solvent (AUC)	Matrix effect (as % of stds in solvent)	Stds in postextraction matrix (pmol)	Stds in extraction solvent (pmol)	Comparative accuracy (as % of stds in solvent)
Pantothenate	0	2000200.00	NA		105.32	NA	
	62.50	2978250.00	1031450.00	94.82	182.39	57.04	135.11
	250.00	5579000.00	3958250.00	90.41	283.53	218.39	81.60
	1000.00	22342500.00	20840000.00	97.61	1156.25	1043.00	100.76
Dephospho CoA	0	0	NA		0.00	NA	
	62.50	30205.00	38385.00	78.69	63.40	77.09	82.25
	250.00	135900.00	156750.00	86.70	267.73	301.91	88.68
	1000.00	550125.00	682450.00	80.61	1098.18	1207.27	90.96
CoA -SH	0	75627.50	NA		81.13	NA	
	62.50	119425.00	51492.50	85.06	130.94	55.22	90.20
	250.00	286650.00	226825.00	93.03	288.80	242.39	85.68
	1000.00	1033325.00	1026500.00	93.30	1053.57	1009.71	96.31
Acetyl CoA	0	3271.00	NA		13.80	NA	
	62.50	14167.50	11860.50	91.87	73.65	59.98	99.77
	250.00	43103.33	40760.00	97.72	216.27	203.50	99.49
	1000.00	194200.00	219600.00	86.94	968.80	1078.55	88.54
Propionyl CoA	0	22780.00	NA		22.73	NA	
	62.50	86710.00	55222.50	115.77	80.58	50.80	113.85
	250.00	264150.00	226025.00	106.79	234.62	215.40	98.37
	1000.00	1036000.00	1143500.00	88.61	938.00	992.67	92.20
Isovaleryl CoA	0	3540.00	NA		3.87	NA	
	62.50	62860.00	58707.50	101.04	65.78	60.87	101.70
	250.00	278800.00	269425.00	103.48	269.16	277.80	96.89
	1000	1198000.00	1189400.00	100.72	1175.00	1134.14	103.60
Malonyl CoA	0	0	NA		0.00	NA	
	62.50	11617.50	10444.75	111.23	59.48	52.99	112.24
	250.00	48592.50	47407.50	102.50	226.30	239.18	94.62
	1000.00	225525.00	258450.00	87.26	1060.25	1177.55	90.04
Succinyl CoA	0	8135.75	NA		90.02	NA	
	62.50	12180.00	4675.00	86.51	162.20	64.24	112.35
	250.00	25990.00	19500.00	91.56	326.15	258.43	91.37
	1000.00	88582.50	74340.00	108.21	1089.95	883.25	113.21

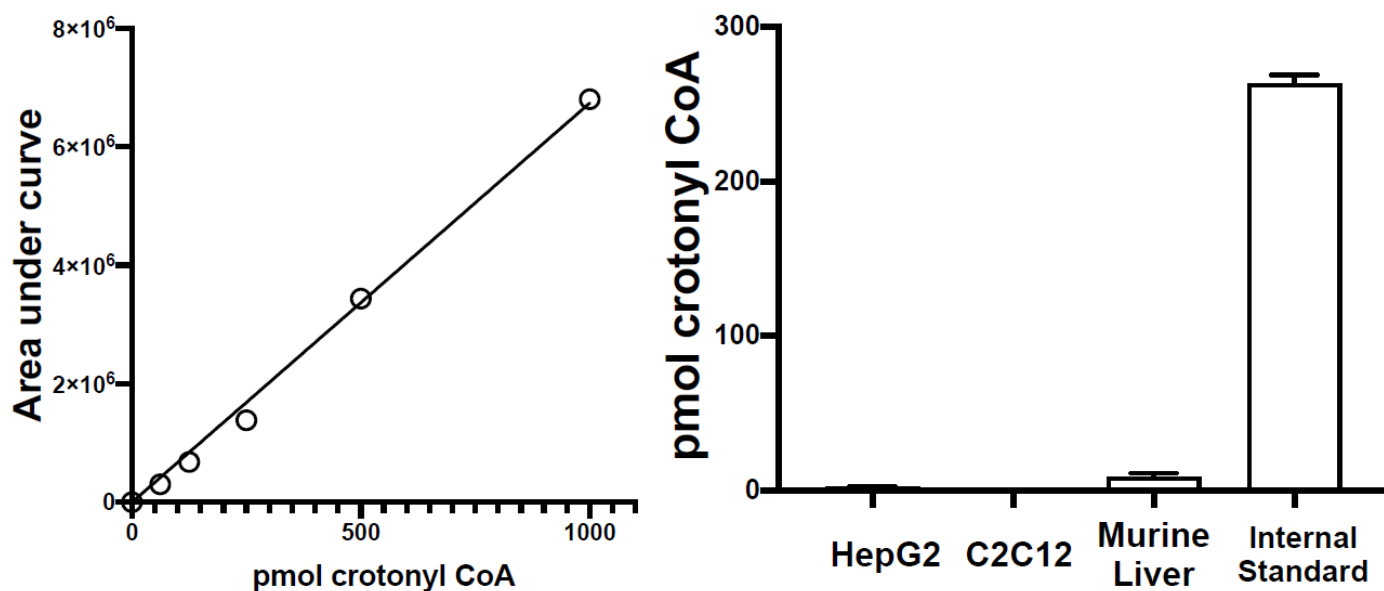


Figure S4. Endogenous crotonoyl CoA concentrations are significantly below the amount of crotonoyl CoA utilized as an internal standard for the proposed method. (*Left*). Increasing concentrations of crotonoyl CoA were spiked into 200 μ L of extraction solution and analyzed via LC-MS/MS. The calibration curve was generated by plotting the area under the curve for crotonoyl CoA against the amount of analyte contained in 200 μ L of extraction solution. (*Right*) The abundance of endogenous crotonoyl CoA was determined for 6×10^6 HepG2 cells, 8×10^8 C2C12 cells, and 5mg murine liver tissue and compared to a blank sample spiked with 1 μ M crotonoyl CoA as an internal standard. Cell lines had endogenous crotonoyl CoA levels less than 1% of the internal standard, and less than 3% for murine liver. Data are presented as mean \pm standard error of the mean (S.E.M.) for n^32 technical replicates.