

Supporting Information

Targeted Determination of Tissue Energy Status by LC-MS/MS

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Table of Contents

Page S3- Supplementary Methods

Page S4 - **Table S1.** Calibration data for various analytes.

Page S5- **Table S2.** Accuracy, recovery and precision for analysis of liver tissue spiked with external standards.

Page S6 - **Table S3.** Intra- and inter-day repeatability of the tissue processing and analytical method for various analytes.

Page S7 - **Figure S1-** Effect of DBAA concentration on A) retention time and B) MS signal. MRM chromatograms of mouse liver extract using DBAA as ion-pairing reagent on a Xbridge C18 column and detection by positive ion mode. C) NAD⁺, D) NADH detected as ADPR, E) AMP, F) ADP, G) ATP, H) Acetyl-CoA, I) Malonyl-CoA, J) Succinyl-CoA, K) Propionyl-CoA.

Page S8 - **Figure S2** – TEA and DMHA ion pairing reagents give poor chromatographic peak shapes for nucleotides. MRM chromatograms of mouse liver extract using 10mM TEA as ion-pairing reagent on a Xbridge C18 column and detection by positive ion mode. A) AMP, B) ADP, C) ATP, D) ADPR and E) NAD. MRM chromatograms of mouse liver extract using 2.5mM DMHA as ion-pairing reagent on a Xbridge C18 column and detection by positive ion mode. F) AMP and G) ADPR.

Page S9- **Figure S3** - Analyte degradation profiles from the neutralized liver extracts stored at room temperature (RT), 4 C, -20 C, or acidified liver extract stored at -80 C.

Page S10 - **Figure S4.** The effect of delayed sample freezing on organic acid levels in mouse liver tissue. Samples were excised and frozen immediately or after variable time delays. Organic acids were measured by GC-MS as described in the supplemental methods. Effects on A) lactate, pyruvate, B) the lactate/pyruvate ratio (as an indicator of NADH/NAD⁺), C) low, D) medium and E) high concentration TCA cycle intermediates were as described in Figure 4A.

Page S11 – **Figure S5.** Detection of free CoA and GTP. MRM chromatograms of mouse liver extract using DBAA as ion-pairing reagent on a Xbridge C18 column and detection by positive ion mode of A) free CoA and B) GTP. MS full scans of C) free CoA and D) GTP with DBAA as ion-pairing reagent in positive ion mode. MS/MS spectra of E) Free CoA (MRM transition of 768/261) and F) GTP (MRM transition of 653/130).

The quantification of organic acids in mouse liver measured by GC-MS

To examine the viability of tissue following dissection, mouse liver was dissected and frozen immediately or after a 10-60 second delay. Organic acids were measured by the Gas chromatography–mass spectrometry (GC-MS) method as described previously¹. Approximately 50 mg of frozen tissue was homogenized in 0.8% sulfosalicylic acid and 5 M hydroxylamine-HCl solution. Samples were spun at 4°C for 10 minutes. The supernatant was neutralized with 2 M KOH to pH 6-7 and then incubated at 65°C for 60 min. The reaction mixture was acidified to pH 1-2, saturated with sodium chloride, and extracted with ethyl acetate. The dried extract was added to acetonitrile and MTBSTFA as silylation reagent and reacted at 60 °C for 60 min. The derivatives were analyzed in both scan and SIM modes by using an Agilent 7890A gas chromatography interfaced to an Agilent 5975C mass-selective detector (70eV, electron ionization source). An HP-5ms GC column (30 m×0.25 mm I.D., 0.25 µm film thickness) was used for all analyses.

References

- (1) Des Rosiers, C.; Fernandez, C. A.; David, F.; Brunengraber, H. J. Biol. Chem. 1994, 269 (44), 27179–27182.

Table S1. Calibration data for analytes.

Analyte	Calibration Curve	r	Linear Range (ng/mL)	Range for Liver (ng/mL)
NAD	$y = 0.6619x + 0.0075$	0.9999	10–150,000	20,000-60,000
ADPR	$y = 0.262x + 0.007$	0.9995	10–150,000	200-3,000
AMP	$y = 0.8013x - 0.0131$	0.9995	10–150,000	1,000-15,000
ADP	$y = 1.2385x + 0.0149$	0.9999	10–150,000	15,000-50,000
ATP	$y = 0.8674x + 0.0015$	0.9996	10–150,000	20,000-100,000
Acetyl CoA	$y = 0.5655x + 0.0098$	0.9997	10–20,000	50-3,000
Malonyl CoA	$y = 0.8659x + 0.0074$	0.9997	10–50,000	10-500
Succinyl CoA	$y = 1.5357x + 0.028$	0.9994	10–50,000	10-2,000
Propionyl CoA	$y = 4.3028x - 0.0272$	0.9998	10–50,000	10-500

Table S2. Accuracy, recovery and precision for analysis of liver tissue spiked with external standards.

Analyte	Spiked 1.5ug			Spiked 4.0 ug			Spiked 6.0 ug		
	Found (μg)	Recovery	CV	Found (μg)	Recovery	CV	Found (μg)	Recovery	CV
NAD ⁺	1.66	110.56	4.80	3.35	83.76	3.00	5.40	89.98	2.17
ADPR (NADH)	1.51	100.79	8.27	3.96	98.88	3.17	5.87	97.85	2.18
AMP	1.49	99.41	3.16	3.99	99.83	2.22	5.74	95.63	1.55
ADP	1.52	101.20	2.32	3.35	83.68	4.01	5.94	98.97	1.81
ATP	1.63	108.79	2.60	3.42	85.38	2.54	5.75	95.84	1.28
Acetyl CoA	1.30	86.88	2.95	3.41	85.27	1.90	5.07	84.44	3.22
Malonyl CoA	1.45	96.56	4.33	4.30	107.38	1.31	5.70	94.94	13.86
Succinyl CoA	1.45	96.41	2.60	4.39	109.66	0.51	5.72	95.31	13.39
Propionyl CoA	1.42	94.95	2.67	4.48	112.00	2.97	5.90	98.32	13.56

Table S3. Intra- and inter-day reproducibility of the tissue processing and analytical method for various analytes.

Analyte	Intra-day 1			Intra-day 2			Intra-day 3			Inter-day 4		
	nmol/g of liver	STDEV	CV	nmol/g of liver	STDEV	CV	nmol/g of liver	STDEV	CV	nmol/g of liver	STDEV	CV
NAD	1056.19	81.06	7.67	1088.15	50.85	4.67	852.40	29.51	3.46	998.91	121.55	12.17
ADPR	63.52	6.95	10.94	74.14	3.73	5.03	74.34	7.20	9.69	70.67	7.57	10.71
AMP	258.24	1.80	0.70	256.39	6.35	2.48	282.38	23.54	8.34	265.67	17.52	6.60
ADP	1476.67	21.80	1.48	1377.40	4.51	0.33	1593.09	83.50	5.24	1482.39	103.00	6.95
ATP	2173.05	54.87	2.53	2556.63	105.16	4.11	2491.02	52.26	2.10	2406.90	189.12	7.86
Acetyl-CoA	66.98	0.42	0.63	52.67	1.13	2.14	52.23	1.31	2.50	57.30	7.32	12.78
Malonyl-CoA	3.62	0.33	9.18	5.32	0.33	6.12	4.05	0.33	8.26	4.33	0.82	18.90
Succinyl- CoA	30.32	1.05	3.46	25.14	1.02	4.06	31.41	2.44	7.76	28.96	3.23	11.16
Propionyl- CoA	1.60	0.09	5.85	1.30	0.01	1.03	1.28	0.01	1.14	1.39	0.16	11.71

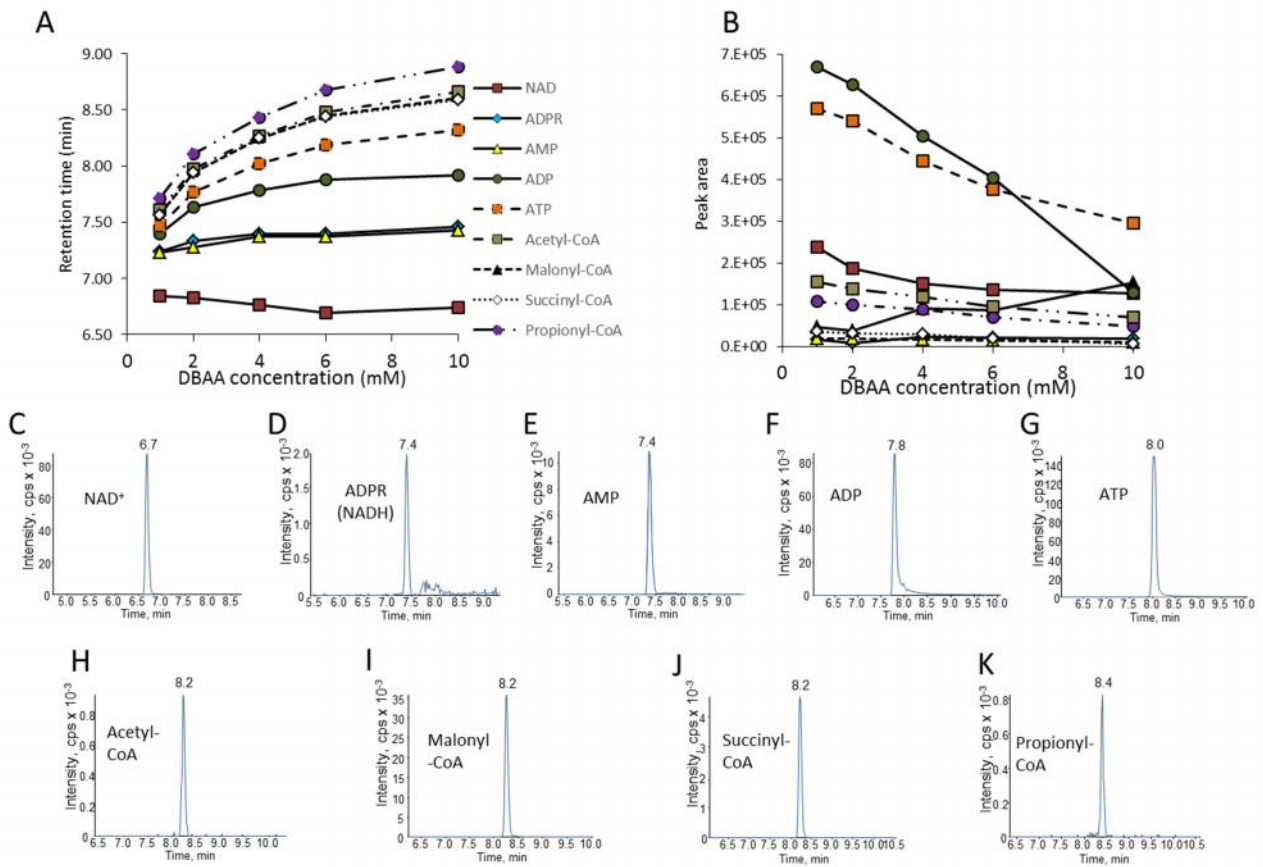


Figure S1- Effect of DBAA concentration on A) retention time and B) MS signal. MRM chromatograms of mouse liver extract using DBAA as ion-pairing reagent on a Xbridge C₁₈ column and detection by positive MRM mode. C) NAD⁺, D) NADH detected as ADPR, E) AMP, F) ADP, G) ATP, H) Acetyl-CoA, I) Malonyl-CoA, J) Succinyl-CoA, K) Propionyl-CoA.

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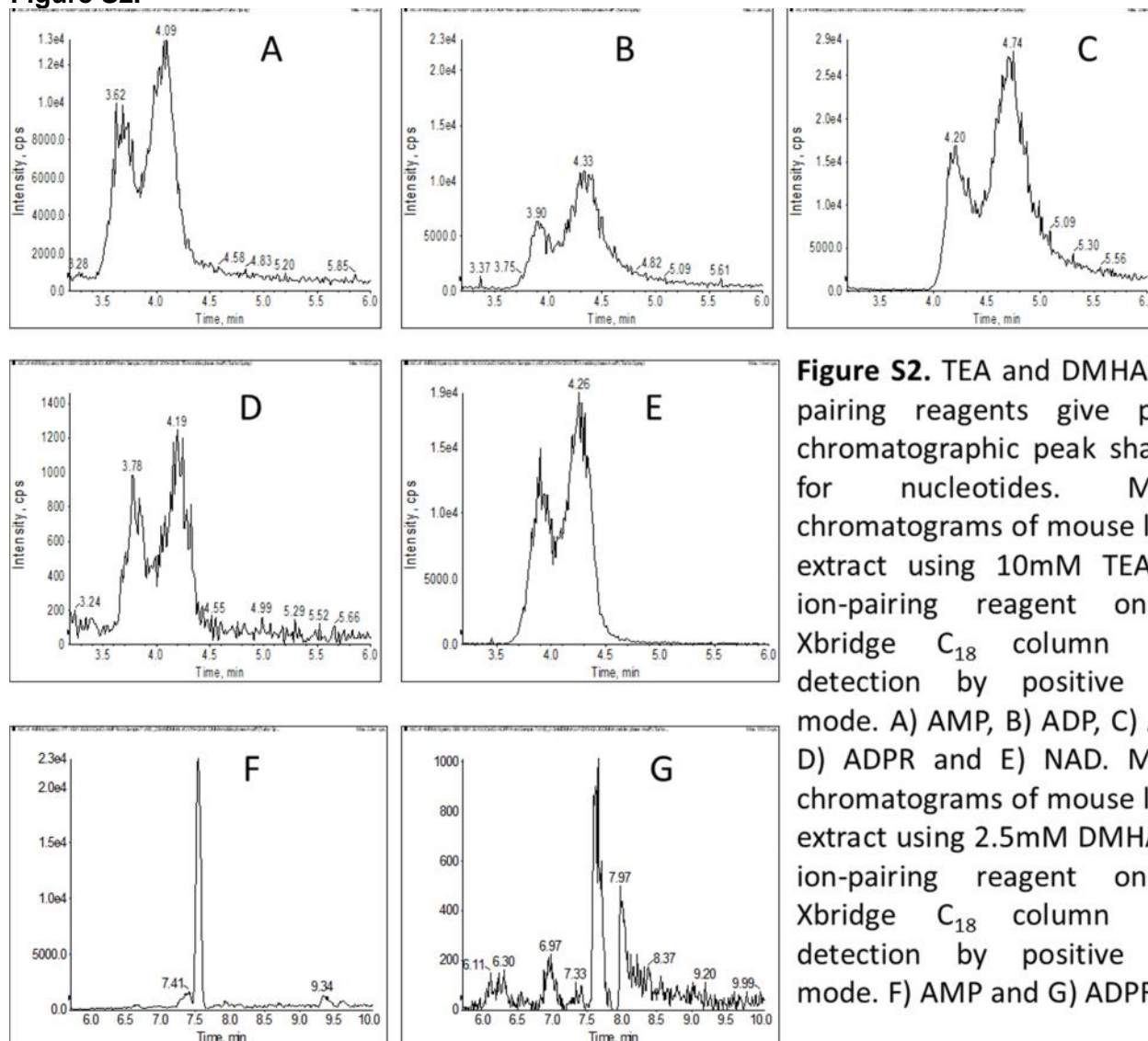


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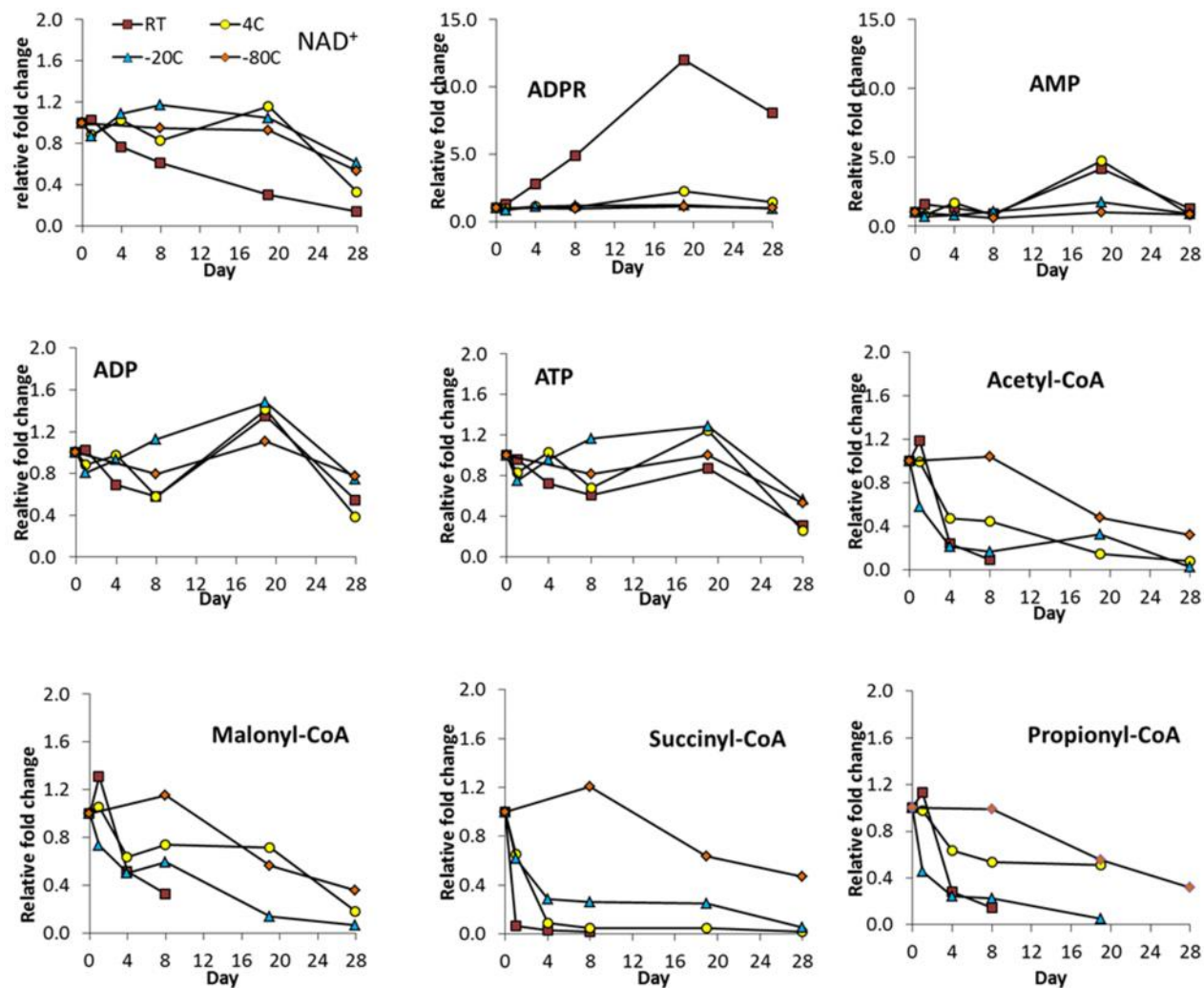


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Figure S4.

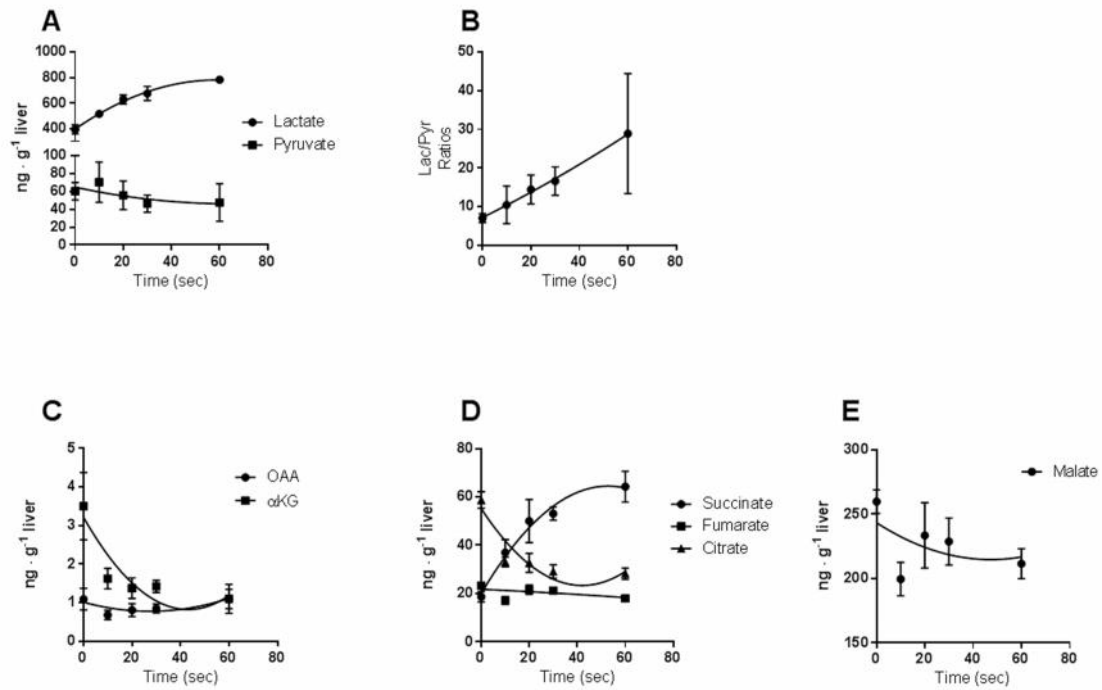


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Figure S5

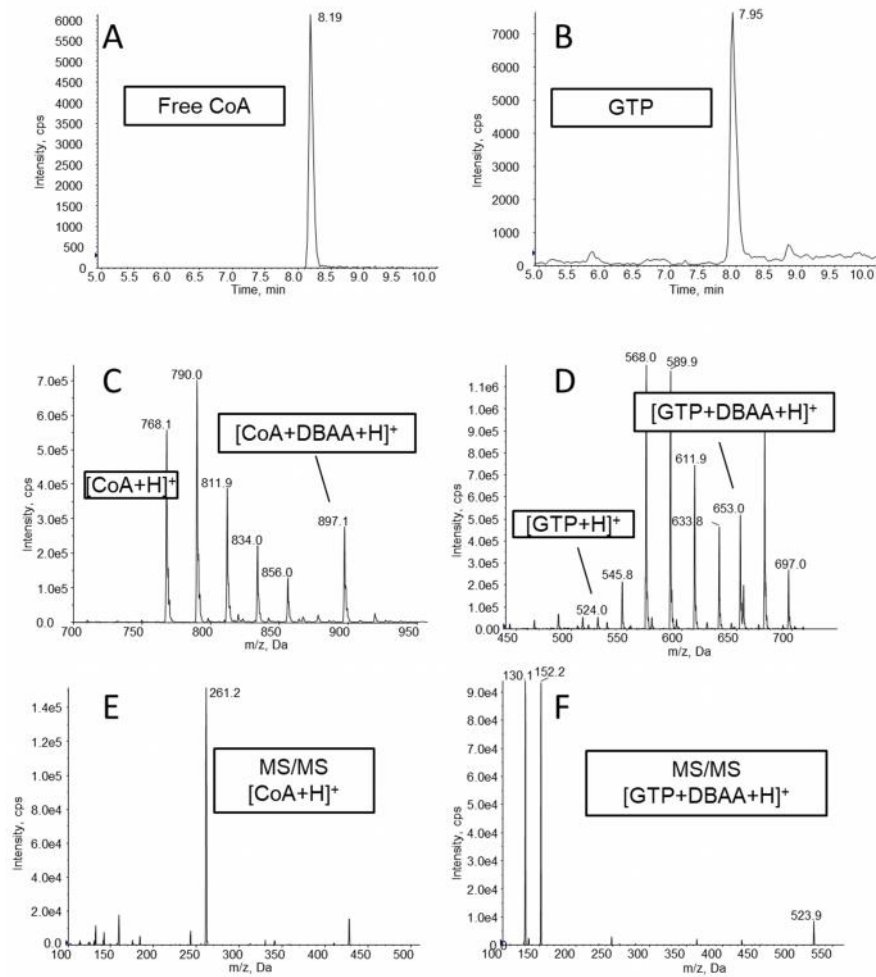


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