Supplementary Materials: Development and validation of a high-throughput mass spectrometry based urine metabolomic test for colorectal cancer screening

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Table S1. Optimized MS parameters for each compound. MRM pair 1 is used for quantitation and MRM pair 2 is for qualification.

Compound	Polarity	Q1	Q3	DP	CE	CXP
Succinic acid 1	-	117.0	73.0	-40	-16	-1
Succinic acid 2	-	117.0	55.1	-40	-22	-7
Succinic acid-D4	-	121.0	77.0	-40	-16	-1
Ascorbic acid 1	-	175.0	114.9	-45	-18	-7
Ascorbic acid 2	-	175.0	86.8	-45	-28	-13
Ascorbic acid-^{13}C	-	176.0	116.0	-45	-18	-7
Carnitine 1	+	162.1	103.1	51	25	6
Carnitine 2	+	162.1	43.2	51	47	6
Carnitine-D9	+	171.0	103.0	51	25	6

Table S2. Extraction recoveries and accuracies for each metabolite.

Metabolite	Recovery (%)[a]	Accuracy (%)[b]
Succinic acid	101.0	110.2
Ascorbic acid	93.8	98.7
Carnitine	93.1	102.7

a. Recovery (%) = (Response (spiked sample))/(Response (post-spiked sample)) x 100

b. Accuracy (%) = (spiked sample-upspiked sample)/(spiked amount) x 100

Table S3. CV% of QC samples for each metabolite within each plate.

	Succinic acid		**Ascorbic acid**		**Carnitine**	
	Average concentration (μM)	CV%	Average concentration (μM)	CV%	Average concentration (μM)	CV%
Kit 1	21.1	14.7%	125.2	8.6%	45.5	9.8%
Kit 2	23.0	5.9%	121.8	8.3%	49.2	10.4%
Kit 3	25.7	16.7%	109.0	10.2%	46.2	8.4%
Kit 4	25.3	5.3%	119.6	4.1%	49.5	5.5%
Kit 5	25.8	10.3%	135.0	7.7%	47.7	4.3%
Kit 6	27.7	13.6%	107.0	4.1%	46.7	6.4%
Kit 7	11.4	13.1%	104.7	14.0%	25.9	10.9%
Kit 8	14.0	11.7%	123.0	4.5%	35.0	6.0%
Kit 9	17.4	12.5%	114.6	9.1%	40.3	11.9%
overall	21.1	10.0%	116.7	6.8%	42.6	7.6%

coefficient of variation (CV%) = (standard deviation)/(mean value)

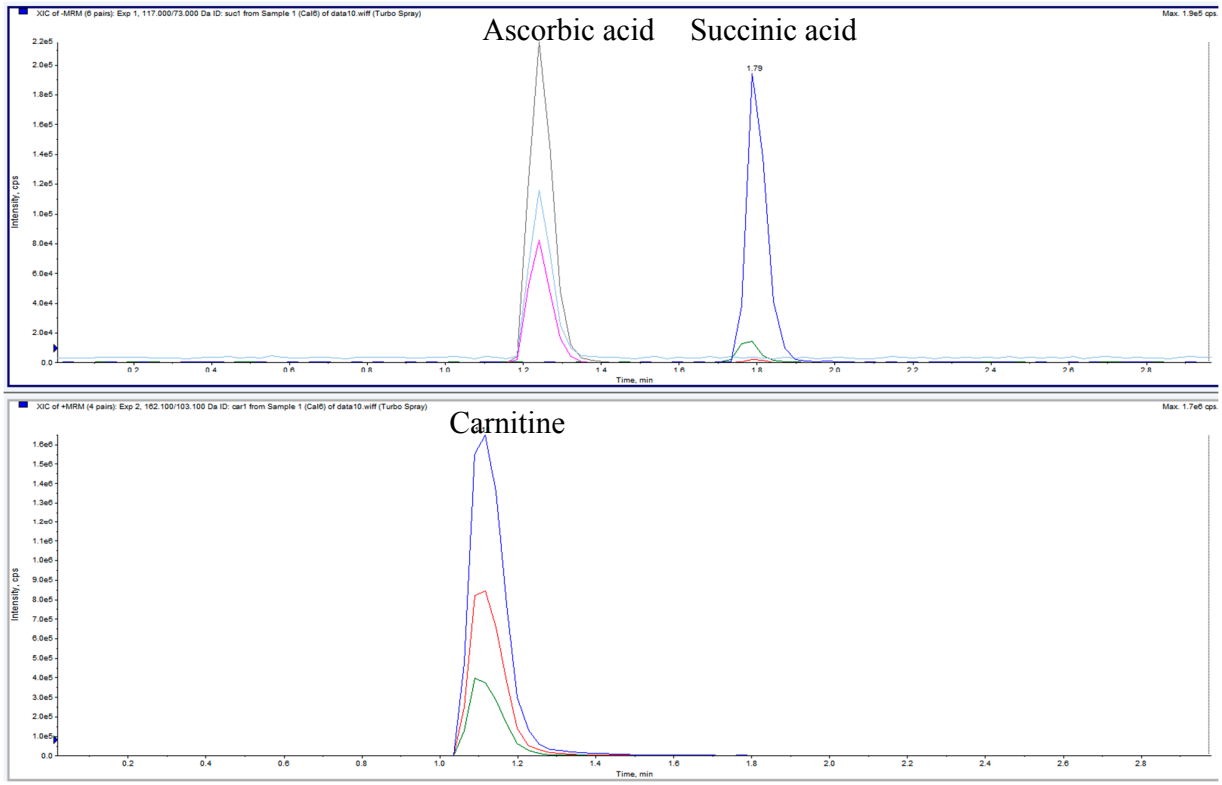

Figure S1. A representative LCMS of Calibrant 6.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Cal7	Urine6	Urine14	Urine21	Urine29	Urine36	Urine44	Urine51	Urine59	Urine66	Urine74
B	ISTD	Cal8	Urine7	Urine15	Urine22	Urine30	Urine37	Urine45	Urine52	Urine60	Urine67	Urine75
C	Cal1	Urine1	Urine8	Urine16	Urine23	Urine31	Urine38	Urine46	Urine53	Urine61	Urine68	Urine76
D	Cal2	Urine2	Urine9	Urine17	Urine24	Urine32	Urine39	Urine47	Urine54	Urine62	Urine69	Urine77
E	Cal3	Urine3	Urine10	Urine18	Urine25	Urine33	Urine40	Urine48	Urine55	Urine63	Urine70	Urine78
F	Cal4	Urine4	Urine11	Urine19	Urine26	Urine34	Urine41	Urine49	Urine56	Urine64	Urine71	Urine79
G	Cal5	Urine5	Urine12	Urine20	Urine27	Urine35	Urine42	Urine50	Urine57	Urine65	Urine72	Urine80
H	Cal6	QC	Urine13	QC	Urine28	QC	Urine43	QC	Urine58	QC	Urine73	QC

Figure S2. A representative plate map. LCMS sequence runs vertically.

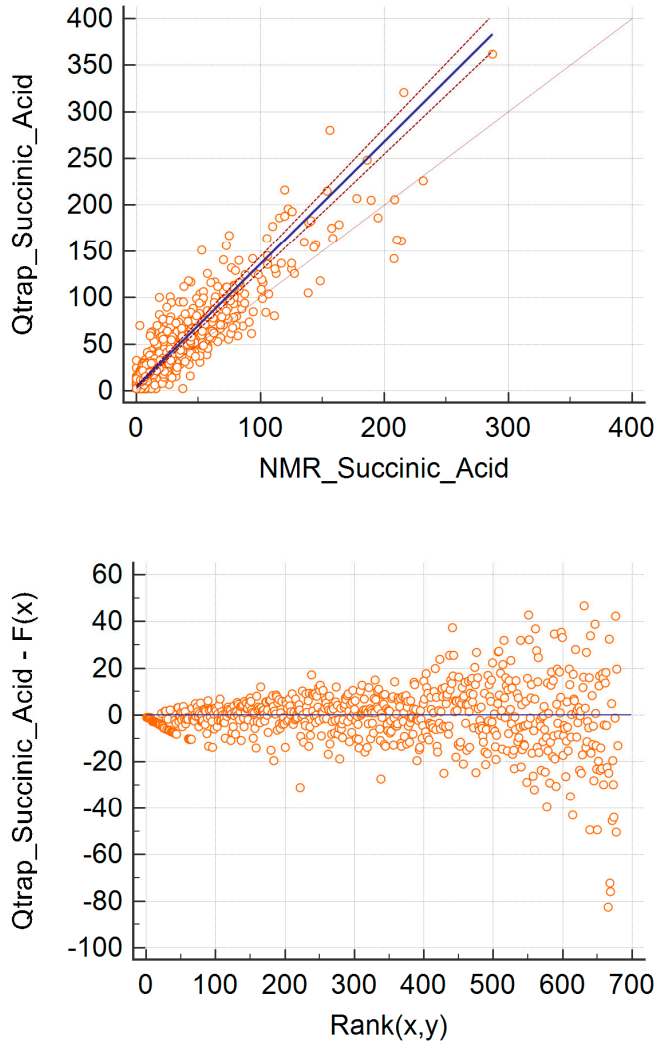

Figure S3. Passing and Bablok regression analyses of MS-quantified on NMR-quantified data for Succinic acid, N = 685; concentration range 0-362 µmol/L; Pearson correlation coefficient r = 0.862, P < 0.0001. (**A**) Scatter diagram with regression line and confidence bands for regression line. Identity line is dashed. Regression line equation: y = 4.17 + 1.32 x; 95% CI for intercept 2.72 to 5.33 and for slope 1.26 to 1.38 indicated small constant and small proportional difference. Cusum test for linearity indicates significant deviation from linearity (P<0.01). (**B**) Residual plot presents distribution of difference around fitted regression line.

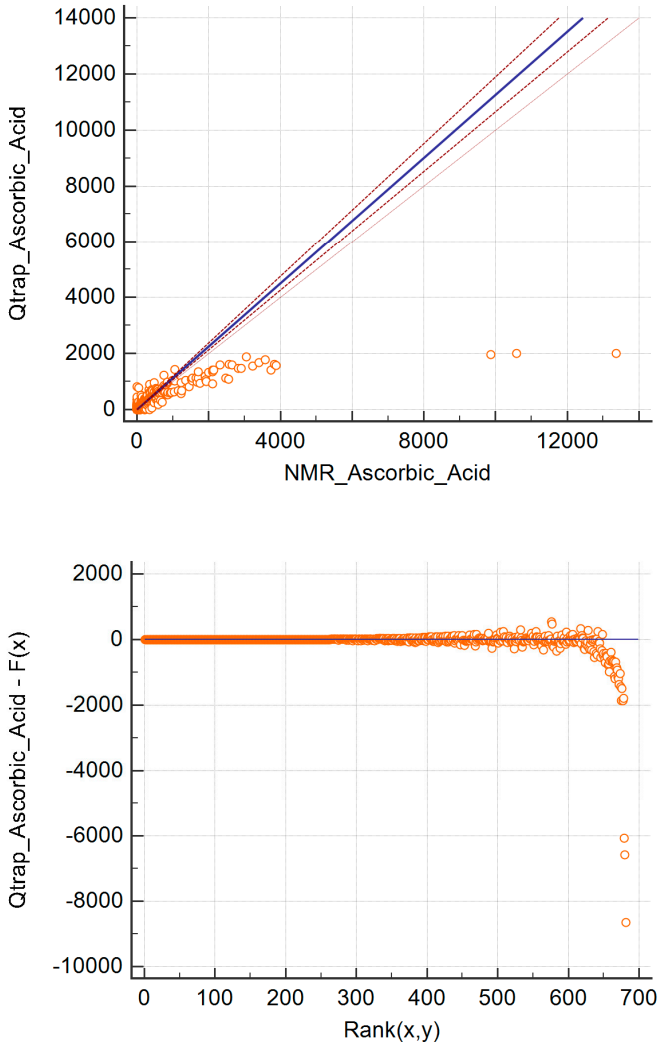

Figure S4. Passing and Bablok regression analyses of MS-quantified on NMR-quantified data for Ascorbic acid, N = 685; concentration range 0-13368 µmol/L; Pearson correlation coefficient r = 0.800, P < 0.0001. (**A**) Scatter diagram with regression line and confidence bands for regression line. Identity line is dashed. Regression line equation: y = 2.50 + 1.12 x; 95% CI for intercept 2.50 to 2.50 and for slope 1.06 to 1.19 indicated small constant and small proportional difference. Cusum test for linearity indicates significant deviation from linearity (P<0.01). (**B**) Residual plot presents distribution of difference around fitted regression line.

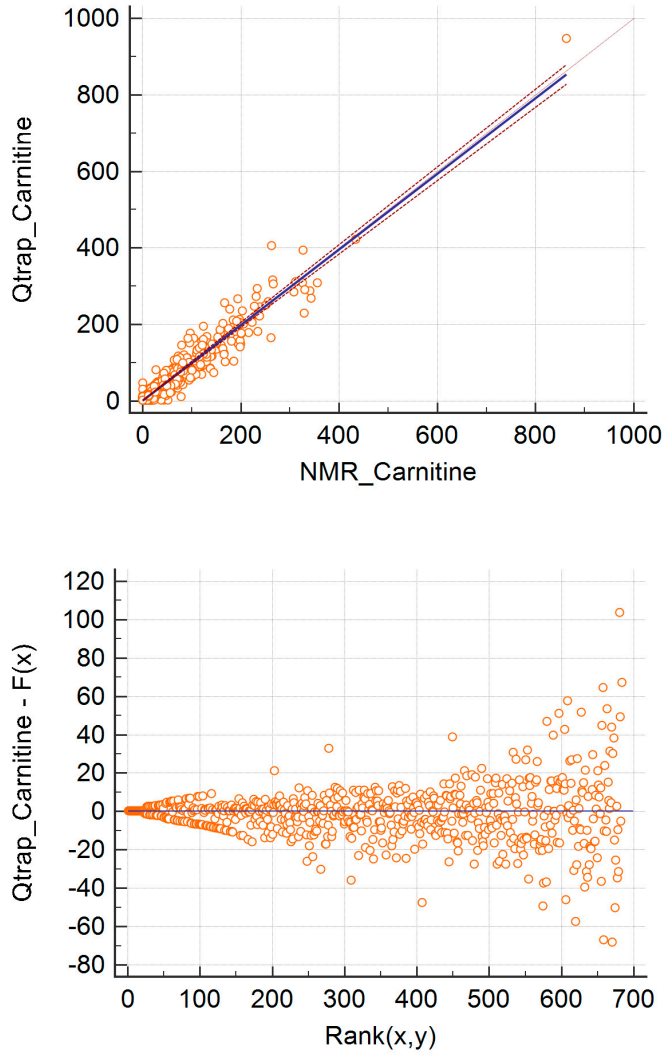

Figure S5. Passing and Bablok regression analyses of MS-quantified on NMR-quantified data for Carnitine, N = 685; concentration range 0-948 µmol/L; Pearson correlation coefficient r = 0.921, P < 0.0001. (**A**) Scatter diagram with regression line and confidence bands for regression line. Identity line is dashed. Regression line equation: y = 1.73 + 0.99 x; 95% CI for intercept 0.77 to 2.50 and for slope 0.96 to 1.02 indicated small constant and small proportional difference. Cusum test for linearity indicates significant deviation from linearity (P=0.04). (**B**) Residual plot presents distribution of difference around fitted regression line.