

Supplementary Information

Certification and long-term stability monitoring of low-content folic acid and 5-methyltetrahydrofolate in human plasma certified reference material by isotope dilution ultra-high performance liquid chromatography/tandem mass spectrometry

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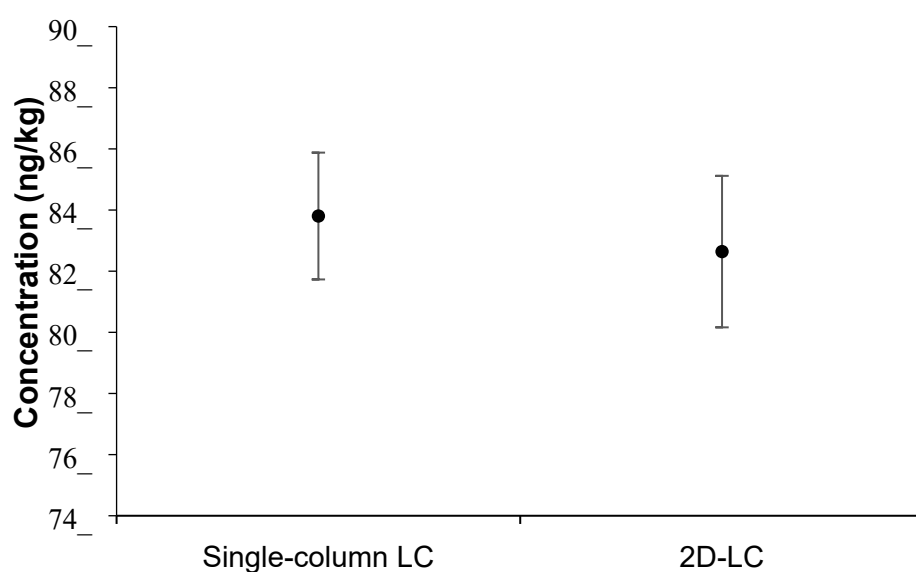


Fig. S1. Comparison of LC/MS measurement results for folic acid in the human plasma CRM using single-column LC with those obtained using 2D-LC. Three CRM sample units were used for the folic acid analysis. The error bar in the figure represents the combined standard uncertainty calculated using Equation 2 in the main manuscript.

Table S1 Standard error of the isotope ratio of folic acid and $^{13}\text{C}_5$ -folic acid in the units of selected human plasma CRM obtained by multiple measurements using LC-MS/MS

Number of CRM units ^a	Run 1	Run 2	Run 3	Run 4	Run 5	Average	Standard error (%)
1	0.919	0.914	0.910	0.905	- ^a	0.912	0.32
181	1.08	1.07	1.03	1.07	0.942	1.04	2.48
437	1.14	1.08	1.13	1.09	1.11	1.11	0.93
500	0.967	0.874	0.927	0.918	0.844	0.906	2.37
662	1.16	1.22	1.13	1.20	1.12	1.17	1.67
855	1.09	1.18	1.16	1.09	1.04	1.11	2.24
1045	1.12	1.11	1.10	1.07	1.03	1.09	1.52
1377	1.16	1.20	1.16	1.18	1.10	1.16	1.49
1539	1.12	1.22	1.23	1.21	1.15	1.18	1.78
1942	1.13	1.17	1.15	1.14	1.17	1.15	0.69

^a Data was not acquired.

Table S2 Results of stability assessments of FA and 5-Me-THF in the human plasma CRM

	Conditions	Values (ng/kg)		
		Average $\pm U_{exp.}^c$	$ x_{CRM} - x_{meas} $	$k\sqrt{u_{CRM}^2 + u_{meas}^2}$
FA ^a	Certification	84.6 \pm 4.26	-	-
	-20 °C, 2 months	76.7 \pm 9.01	7.9	9.5
	-70 °C, 6 months	82.9 \pm 10.2	1.7	11
	-70 °C, 12 months	76.1 \pm 15.0	8.6	22
	-70 °C, 29 months	83.9 \pm 8.94	0.76	8.0
	-70 °C, 58 months	78.3 \pm 9.57	1.6	12
	Conditions	Values (μ g/kg)		
		Average $\pm U_{exp.}^c$	$ x_{CRM} - x_{meas} $	$k\sqrt{u_{CRM}^2 + u_{meas}^2}$
5-Me-THF ^b	Certification	5.80 \pm 0.47	-	-
	-20 °C, 2 months	5.73 \pm 0.13	0.073	0.47
	-70 °C, 5 months	5.76 \pm 0.15	0.037	0.47
	-70 °C, 12 months	5.97 \pm 0.15	0.16	0.48
	-70 °C, 24 months	5.86 \pm 0.15	0.059	0.47
	-70 °C, 48 months	5.82 \pm 0.23	0.088	0.24

^a Folic acid^b 5-Methyltetrahydrofolate^c Expanded uncertainty at 95% of confidence level