

Supplemental Text 1.

This supplemental text provides additional information on the preparation of MeFox¹ calibrators and on instrumentation used.

Stock I solutions of MeFox and ¹³C₅-MeFox (300 mg/L) were prepared in 0.1 mol/L sodium hydroxide (pH 12.4) and the concentration was determined by UV spectrophotometry at 280 nm using a molar absorption coefficient of 19,365 L/(mol × cm) (personal communication with Jean-Pierre Knapp at Merck Cie, March 2012). Aliquots of a 100 mg/L stock II solution in degassed water were stored at -70°C. Aliquots of a 20 μmol/L stock III solution in 0.1 % (w/v) ascorbic acid were prepared every 2 mo, stored at -70°C and used to prepare daily working solutions for calibration.

We used an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) coupled to an API 4000 (chromatographic separation method 2) or API 5500 (mass separation method 3) tandem mass spectrometer (AB Sciex, Foster City, CA) with an electrospray ion source (Turbo Ion SprayTM) in positive ionization mode for sample analysis. The table below specifies the retention time and instrument settings for unlabeled and ¹³C₅-labeled 5-formylTHF and MeFox on the API 5500. General instrument parameters used for LC-MS/MS detection and quantitation of all folate analytes in positive ion multiple reaction monitoring (MRM) mode were as follows: resolution Q1 and Q3: unit; dwell time: 110 ms; ion spray voltage: 5500 V; source temperature: 550°C; curtain gas: 35 psi²; gas 1: 55 psi; gas 2: 60 psi; CAD gas: 8.0 psi.

¹ Mefox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methyltetrahydrofolate; 5-formylTHF, 5-formyltetrahydrofolate.

² Conversion from conventional psi (lb/in²) to SI Pa (N/m²) units: 1 psi = 6.8948 × 10³ Pa

Online Supporting Material

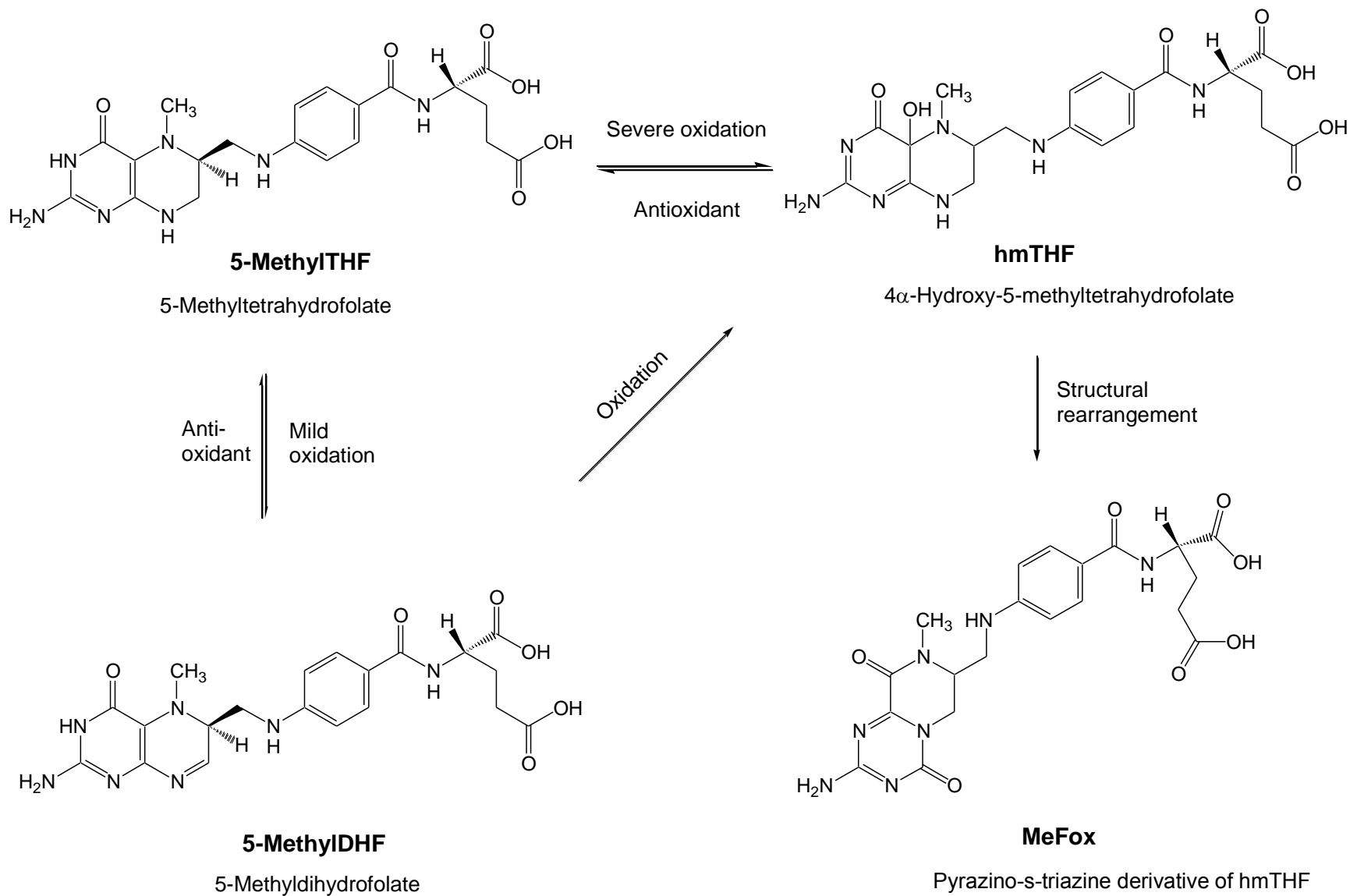
Supplemental Text 1, continued

Analyte	Transition, m/z	T_r ¹ , min	DP, V	CE, V	CXP, V	EP, V
5-FormylTHF	474.4 → 327.2	2.99	135	33	10	10
	474.4 → 299.2 ²	2.96	130	45	10	10
¹³C₅-5-FormylTHF	479.4 → 327.2	2.99	140	31	10	10
	479.4 → 299.2 ²	2.96	130	44	10	10
MeFox	474.4 → 327.2	3.02	135	33	10	10
	474.4 → 284.2 ²	3.02	135	52	10	10
¹³C₅-MeFox	479.4 → 327.2	3.02	140	31	10	10
	479.4 → 284.2 ²	3.02	136	51	10	10

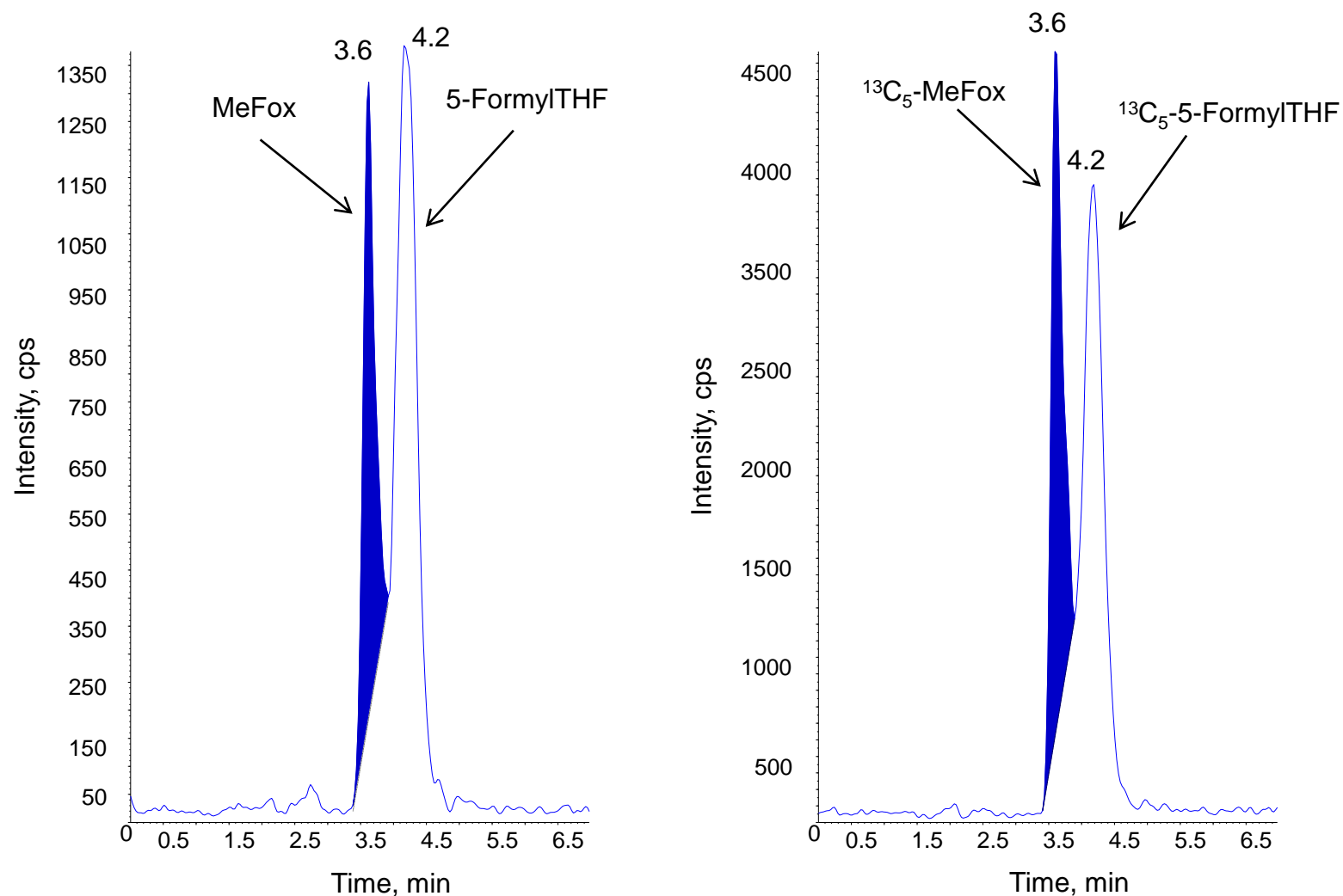
¹ T_r , retention time; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential; EP, entrance potential.

² Transition used for quantitation.

Supplemental Figure 1: Chemical structures of 5-methyltetrahydrofolate and its oxidation products

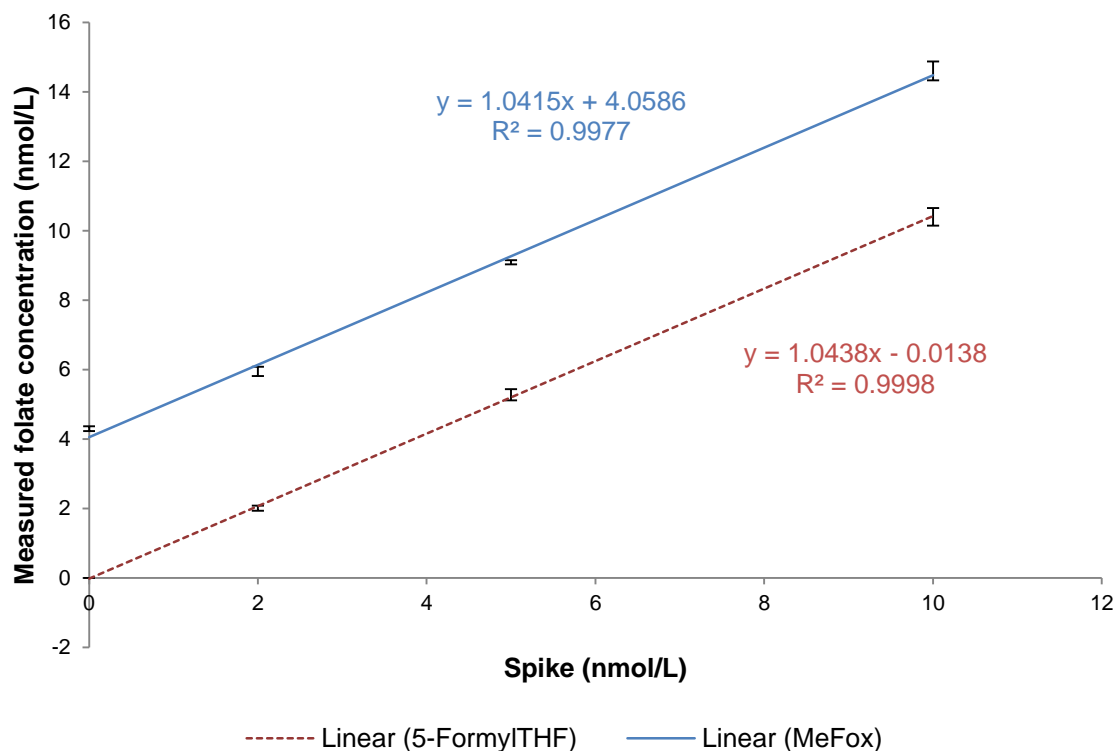


Supplemental Figure 2: Typical LC-MS/MS chromatogram for method 2 showing nearly-baseline separation of 5-formylTHF and MeFox (m/z 474 \rightarrow 327) in the serum high QC pool sample.



The serum QC pool was spiked with 5-formyltetrahydrofolate (5-formylTHF, 3.5 nmol/L) at the time of preparation. The measured MeFox concentration was 2.7 nmol/L. MeFox is the pyrazino-s-triazine derivative of 4 α -hydroxy-5-methyltetrahydrofolate, a stable oxidation product of 5-methyltetrahydrofolate.

Supplemental Figure 3: Ordinary linear regression between 5-formylTHF and MeFox spiking level and measured folate concentrations in a spiked serum specimen



Serum specimen was spiked with three levels (2, 5, and 10 nmol/L) of 5-formylTHF (5-formyltetrahydrofolate) and MeFox (pyrazino-s-triazine derivative of 4 α -hydroxy-5-methyltetrahydrofolate) and samples were processed by solid-phase extraction for LC-MS/MS analysis by method 3 (n = 3 replicates per spike level). Error bars represent the SD of the mean recovery from the 3 replicates.