Supporting Information

Is the stability of folates in dried blood microsamples sufficient to perform home-sampling studies?

Liesl Heughebaert and Christophe Pol Stove*

Laboratory of Toxicology, Department of Bioanalysis, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

*Corresponding author: Christophe.Stove@UGent.be, +32 (0) 9 264 81 35

Supplementary Information Content

1. Supplemental Tables

Table S-1. Multiple reaction monitoring transitions and compound-specific MS parameters for all folate vitamers measured including the internal standards, CE: collision energy.

	Precursor ion (m/z)	Product ion (m/z)	Cone (V)	CE (eV)
5MTHF	460.28	180.13	20	34
		313.20	20	16
5MTHF - ^{13}C5	465.30	180.13	20	34
		313.20	20	16
MeFOX	474.30	283.90	25	35
		327.20	25	20
MeFOX - ^{13}C5	479.40	284.40	25	35
		327.20	25	20
THF	446.24	166.15	4	44
		299.23	4	18
THF - ^{13}C5	451.10	166.15	4	44
		299.30	4	18
5,10CH$^+$THF	456.24	282.19	4	50
		412.66	4	30
5,10CH$^+$THF - ^{13}C5	461.20	282.19	4	50
		416.00	4	30
10FoFA	470.18	176.10	26	42
		295.17	26	24
5FoTHF - ^{13}C5	479.00	166.15	4	44
		327.20	4	18

Table S-2. Spiked concentrations used for the selectivity and matrix effects experiments.

Concentration (nM)	5MTHF	MeFOX	THF	5,10CH$^+$THF	10FoFA
low	450	65	30	30	10
high	900	375	375	375	375

Table S-3. Ion ratios obtained in neat solvent and fresh whole blood matrix (both VAMS samples and DBS dried for 2.5 h) for the five different folate vitamers (mean (CV, %), n = 6).

	5MTHF	MeFOX	10FoFA	5,10CH⁺THF	THF
Neat solvent	0.37 (2%)	0.20 (16%)	0.52 (5%)	0.39 (10%)	0.31 (4%)
Matrix (VAMS)	0.34 (2%)	0.20 (11%)	0.47 (5%)	0.38 (8%)	0.28 (5%)
Matrix (DBS)	0.36 (3%)	0.18 (14%)	0.49 (5%)	0.38 (6%)	0.32 (3%)

Table S-4. Absolute and internal standard (IS) – corrected matrix effects (ME) for 5MTHF, MeFOX, 10FoFA, 5,10CH$^+$THF and THF in VAMS samples and DBS (mean (CV, %). ME were assessed in fresh samples (processed immediately after drying, t_0) and in aged samples (processed after storage at 37 °C for 1 week) with n = 8 per condition.

|	VAMS - fresh				VAMS - aged				DBS - fresh				DBS - aged			
	absolute		IS-corrected		absolute		IS-corrected		absolute		IS-corrected		absolute		IS-corrected	
	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high
5MTHF	115 (9%)	115 (7%)	101 (6%)	102 (6%)	120 (7%)	120 (7%)	102 (4%)	100 (5%)	103 (6%)	104 (8%)	101 (3%)	101 (5%)	104 (6%)	106 (5%)	99 (4%)	102 (4%)
MeFOX	56 (9%)	62 (10%)	100 (6%)	103 (4%)	29 (7%)	31 (7%)	103 (4%)	106 (5%)	31 (11%)	32 (10%)	102 (5%)	104 (5%)	29 (8%)	31 (6%)	102 (5%)	104 (4%)
10FoFA	120 (12%)	121 (10%)	104 (5%)	105 (6%)	54 (10%)	56 (11%)	105 (6%)	107 (6%)	98 (19%)	94 (19%)	104 (6%)	102 (5%)	49 (11%)	49 (10%)	109 (8%)	111 (5%)
5,10CH$^+$THF	121 (10%)	123 (7%)	95 (9%)	97 (7%)	134 (14%)	131 (11%)	97 (11%)	96 (9%)	116 (12%)	111 (7%)	100 (8%)	101 (5%)	118 (7%)	110 (7%)	99 (7%)	94 (7%)
THF	101 (10%)	102 (6%)	101 (4%)	99 (6%)	82 (11%)	84 (9%)	88 (5%)	87 (6%)	86 (10%)	86 (7%)	98 (5%)	96 (5%)	70 (10%)	73 (7%)	86 (7%)	87 (6%)

2. Supplemental Figures
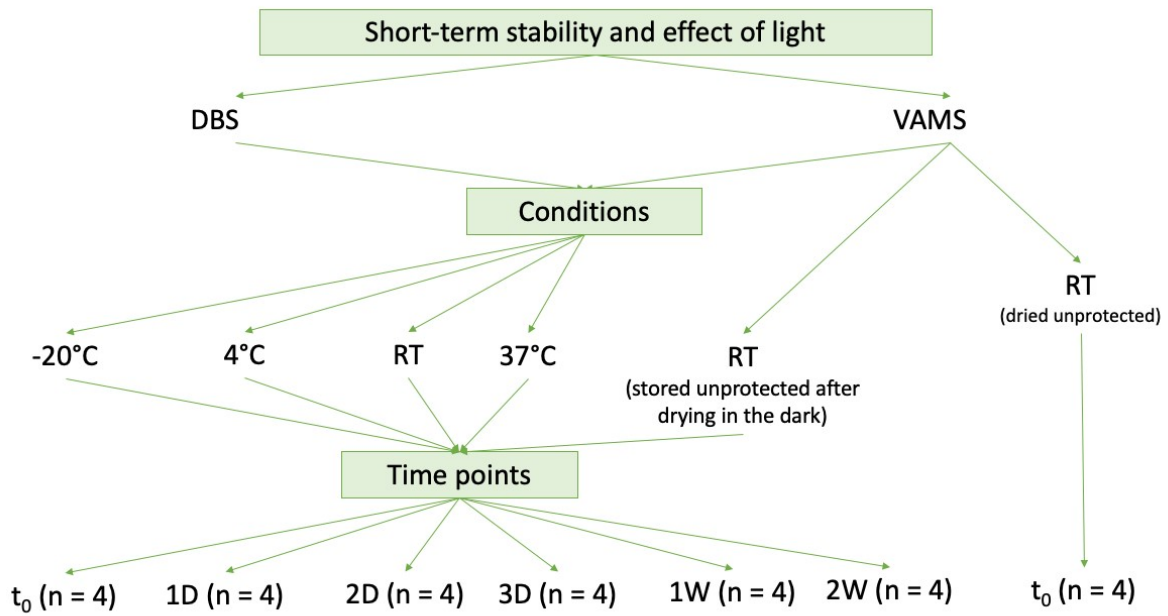
Fig. S-1 Schematic overview of the short-term stability and effect of light experiment (n = 4 technical replicates). Dried blood spots (DBS) and volumetric absorptive microsamples (VAMS) were processed and analyzed in separate analytical runs. Abbreviations: RT: room temperature, t_0: baseline condition, 1D: one day storage, 2D: two day storage, 3D: three day storage, 1W: one week storage, 2W: two week storage.
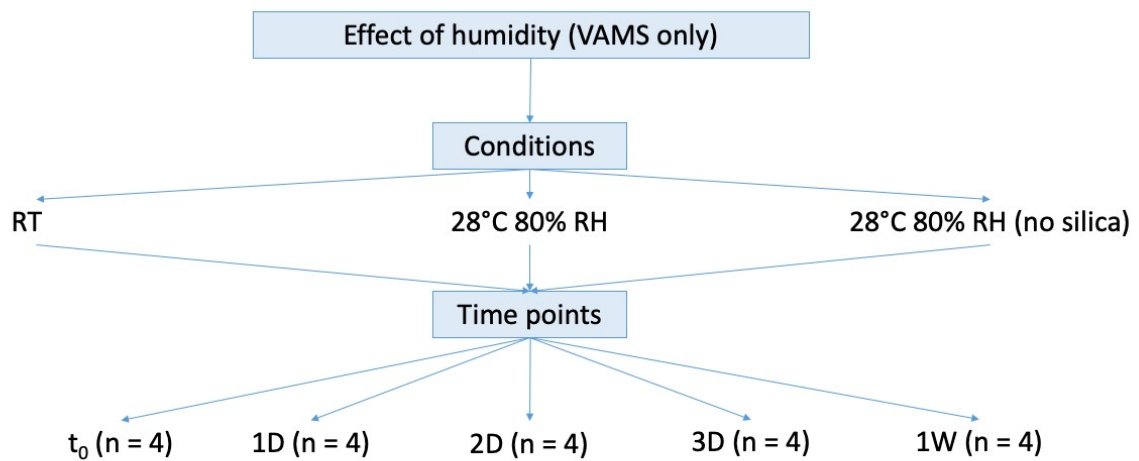

Fig. S-2 Schematic overview of the effect of humidity experiment (n = 4 technical replicates). Abbreviations: RH: relative humidity, RT: room temperature, t_0: baseline condition, VAMS: volumetric absorptive microsampling, 1D: one day storage, 2D: two day storage, 3D: three day storage, 1W: one week storage.
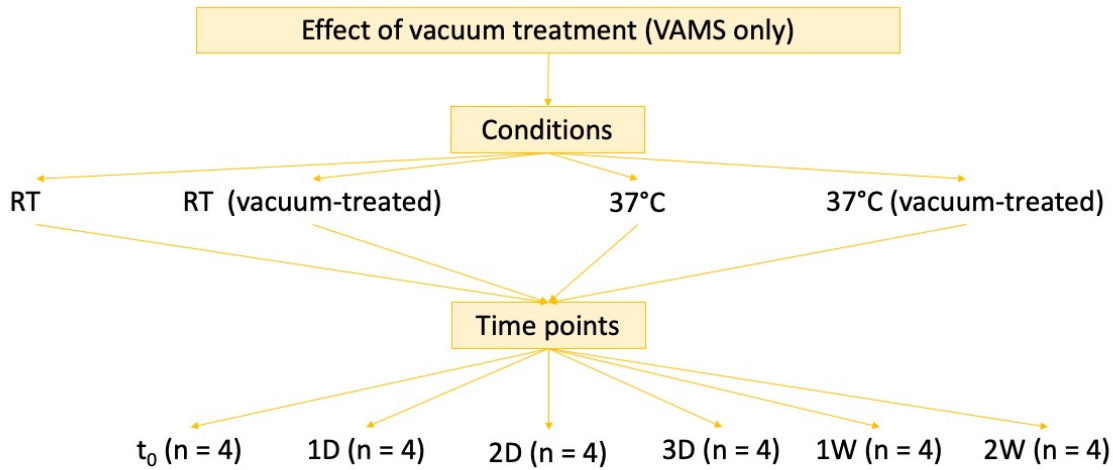
Fig. S-3. Schematic overview of the effect of vacuum treatment experiment (n = 4 technical replicates). Abbreviations: RT: room temperature, t_0: baseline condition, VAMS: volumetric absorptive microsampling, 1D: one day storage, 2D: two day storage, 3D: three day storage, 1W: one week storage, 2W: two week storage.
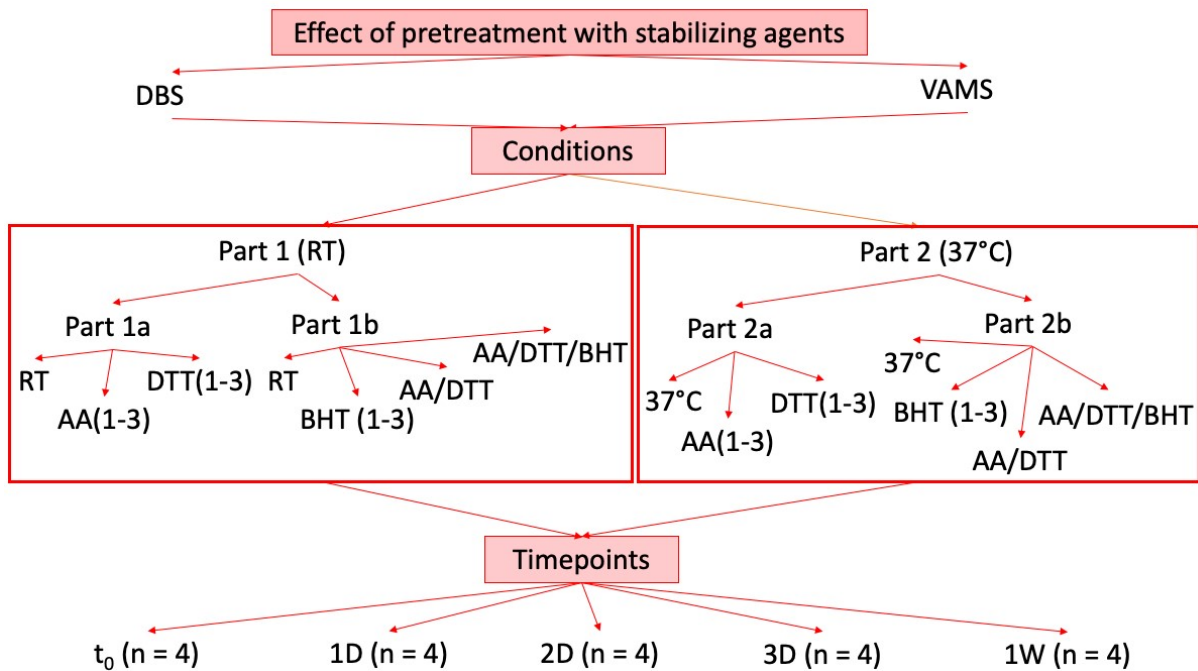

Fig. S-4. Schematic overview of the effect of stabilizing agents experiments. For practical reasons, dried blood spots (DBS) and volumetric absorptive microsamples (VAMS) were processed and analyzed in separate analytical runs and also room temperature (RT) and 37 °C samples needed to be processed separately (part 1 and part 2, respectively) as well as a few of the stabilizing agents (part 1a-b and part 2a-b, respectively). Abbreviations: t_0: baseline condition, 1D: one day storage, 2D: two day storage, 3D: three day storage, 1W: one week storage, AA(1-3): three different concentrations of ascorbic acid, DTT(1-3): three different concentrations of DL-dithiothreitol, BHT(1-3): three different concentrations of butylated hydroxytoluene, AA/DTT: mixture of ascorbic acid and DL-dithiothreitol and AA/DTT/BHT: mixture of ascorbic acid, DL-dithiothreitol and butylated hydroxytoluene.
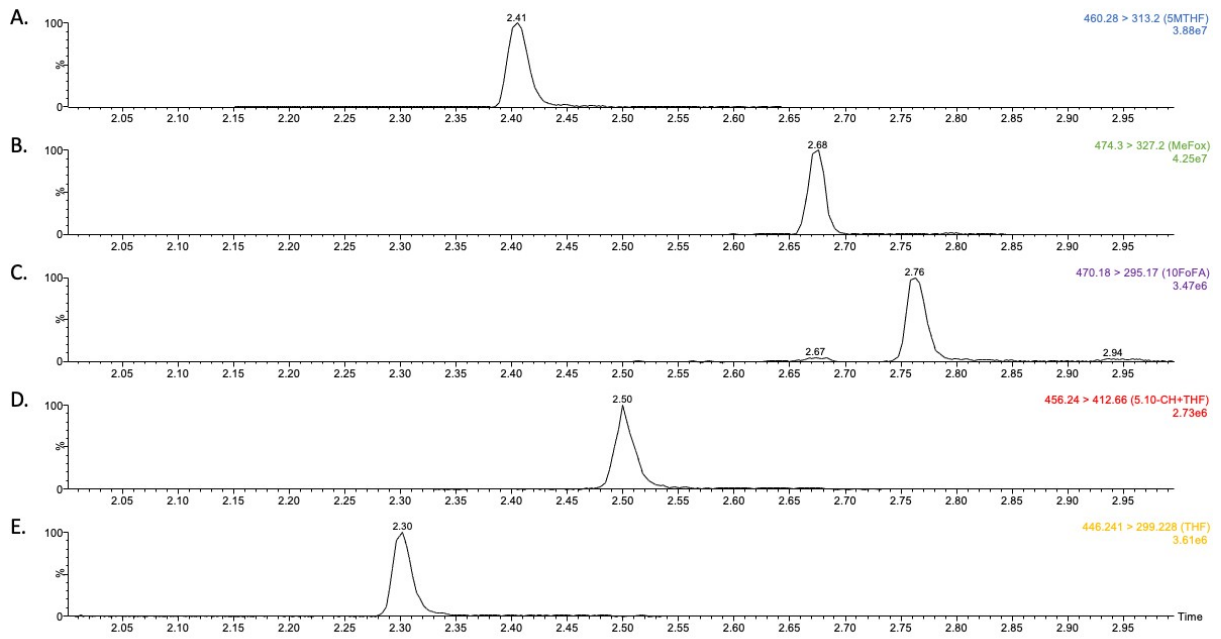
Fig. S-5. Chromatographic separation of the five monitored folates in an authentic blood sample dried on a volumetric absorptive microsampling (VAMS) device. 5-methyltetrahydrofolate (A), MeFOX (B), 10-formylfolic acid (C), 5,10-methenyltetrahydrofolate (D) and tetrahydrofolate (E).