

## **Analytical and Bioanalytical Chemistry**

### **Electronic Supplementary Material**

#### **High-throughput and simultaneous quantitative analysis of homocysteine–methionine cycle metabolites and co-factors in blood plasma and cerebrospinal fluid by isotope dilution LC–MS/MS**

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**Table S1** List of analyzed compounds and internal standard (IS) stock solutions using same dilution solutions. These solutions were stored at -20°C until use for 3 months

<b>Analyte</b>	<b>Targeted concentration(<math>\mu\text{g/mL}</math>)</b>	<b>Concentration and solution type</b>
homocysteic acid	2500.0	0.1 M HCl
taurine	2500.0	MeOH/H <sub>2</sub> O (10/90)
serine	2500.0	0.1 M HCl
cystine	2500.0	1 M HCl
glycine	2500.0	0.1 M HCl
homocystine	2500.0	0.1 M NaOH
riboflavin	250.0	0.1 M NaOH
methionine	2500.0	0.1 M HCl
pyridoxine	250.0	0.1 M HCl
cystathionine	250.0	0.1 M HCl
SAH	250.0	0.1 M HCl
pyridoxamine	250.0	0.1 M HCl
SAM	250.0	0.1 M HCl
DMG	2500.0	MeOH/H <sub>2</sub> O 10/90
betaine	2500.0	MeOH/H <sub>2</sub> O 10/90
choline	2500.0	MeOH/H <sub>2</sub> O 50/50
5-MTHF	250.0	NH <sub>4</sub> OAc 10 mmol/L, ascorbic acid 10 %, DTT 2% in H <sub>2</sub> O

**Table S2** Working range of all metabolites for plasma and CSF analyses

<b>Analyte</b>	<b>Conc. Range</b>		<b>Units</b>
Homocysteic acid	2	- 250	μM
taurine	4	- 500	μM
serine	4	- 500	μM
cystine	2	- 250	μM
glycine	4	- 500	μM
homocystine	2	- 250	μM
riboflavin	4	- 500	nM
methionine	2	- 250	μM
pyridoxine	2	- 250	nM
cystathionine	4	- 500	nM
SAH	8	- 1000	nM
pyridoxamine	2	- 250	nM
SAM	8	- 1000	nM
DMG	0.8	- 100	μM
betaine	2	- 250	μM
choline	0.8	- 100	μM
5-MTHF	8	- 1000	nM

**Table S3** Internal standard (IS) working solution concentrations for spiking

<b>Analyte</b>	<b>Concentration</b>	<b>Units</b>
homocysteic acid_d4	1000	μM
taurine_ <sup>13</sup> C <sub>2</sub>	2000	μM
serine-d <sub>3</sub>	2000	μM
cystine_d4	1000	μM
glycine_d2	2000	μM
homocystine_d8	1000	μM
riboflavin_ <sup>13</sup> C <sub>4</sub> <sup>15</sup> N <sub>2</sub>	2	μM
methionine_d3	1000	μM
pyridoxine_d2	1	μM
cystathionine_d4	2	μM
SAH_d4	4	μM
pyridoxamine_d3	1	μM
SAM_d3	4	μM
DMG_d6	400	μM
betaine_d11	1000	μM
choline_d9	400	μM
5-MTHF_ <sup>13</sup> C <sub>5</sub>	4	μM

**Table S4** Summary of HPLC settings

Injection volume	10 $\mu$ L		
LC column temperature	Room temperature		
Mobile Phase	A – 5 mM PFHA in water B – Acetonitrile		
Pre clean needle wash 1	ACN/H <sub>2</sub> O (50/50; v/v)		
Post clean needle wash 2	ACN/H <sub>2</sub> O (90/10; v/v)		
Mobile phase flow	0.4 mL/min		
Gradient	Time (min)	% A	% B
	0.0	95	5
	0.5	95	5
	8	5	95
	10.0	5	95
	11.0	95	5
	13.0	95	5
Total run time (min)	13.0 min		

**Table S5** Summary of MS settings

Source type	ESI+
Spray voltage	4 kV
Vaporizer temperature	200 °C
Sheath gas pressure	10 units
Ion sweep gas pressure	0 units
Auxiliary gas pressure	5 units
Capillary temperature	350 °C
S-lens RF	used tune S-lens values
CID gas	Argon, quality 5.0, 1.5 mTorr
Scan width	0.5 s
Scan time	0.02 s

**Table S6** Matrix effects % (ME) estimated in spiked plasma and CSF samples with labeled internal standards

<b>Analyte</b>	<b>ME % in plasma</b>	<b>ME % in CSF</b>
homocysteic acid	120.2	32.1
taurine	105.9	10.6
serine	-11.4	-24.5
total cysteine	2.4	-22.7
glycine	-10.1	-19.7
total homocysteine	6.5	-7.0
riboflavin	25.6	-3.0
methionine	-2.8	7.3
pyridoxine	-11.9	-8.6
cystathionine	-0.5	-9.1
SAH	-6.4	-3.8
pyridoxamine	0.7	-19.6
SAM	-3.0	-6.3
DMG	-14.4	-30.0
betaine	-15.1	-26.3
choline	-35.7	-23.2
5-MTHF	-73.3	-36.9