Quantification of cellular folate species by LC-MS after stabilization by derivatization

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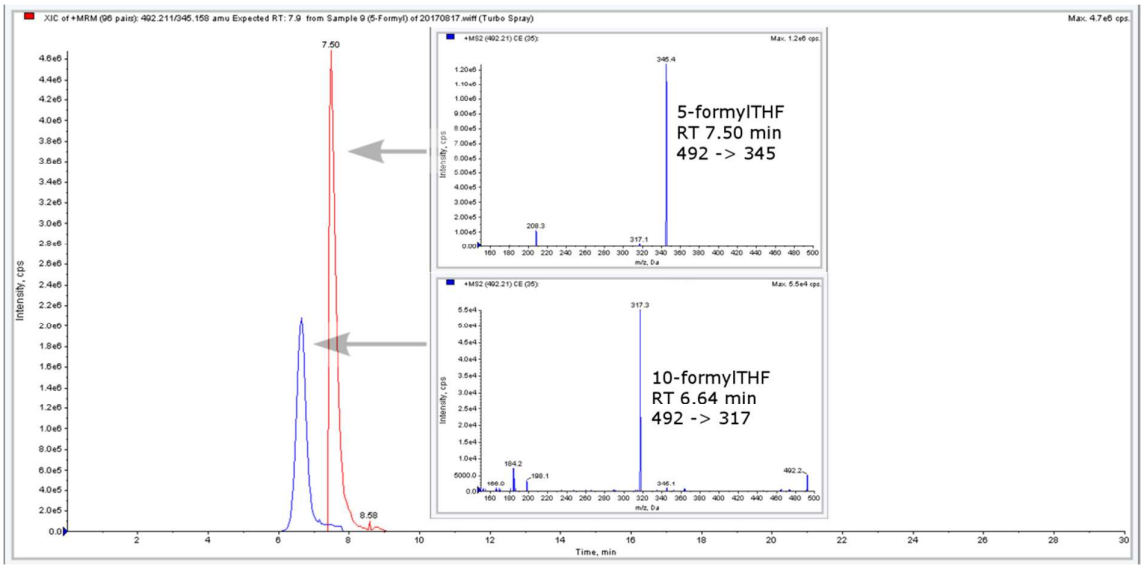

Fig. S1: Chromatographic separation and specific fragmentation patterns of 5- and 10-formyTHF-Glu1.

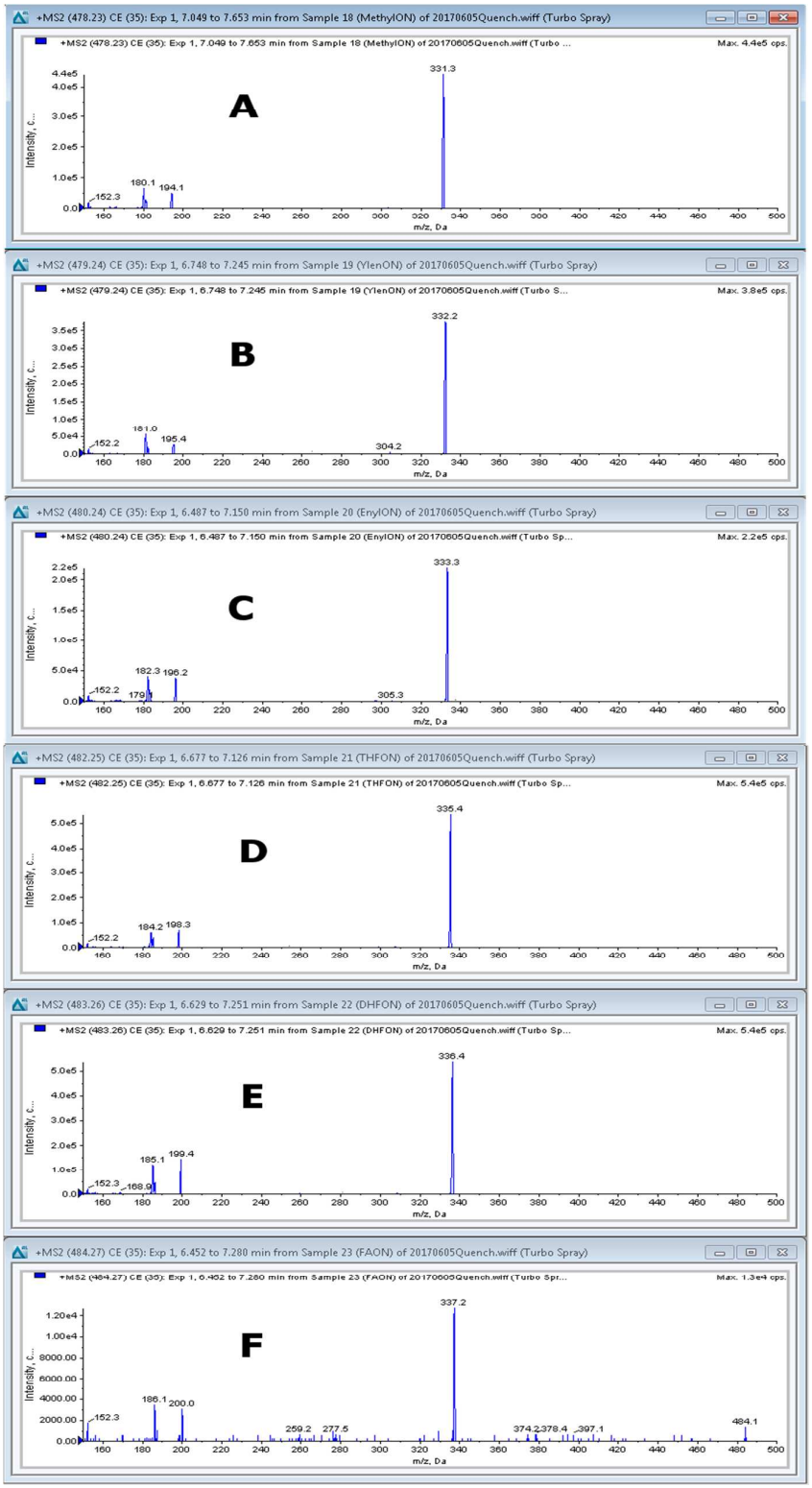

Fig. S2: Fragment spectra of isotopologues of 5,10-dimethylTHF-Glu1 in ascending m/z order. A: derivative of 5-methylTHF-Glu1; B: derivative of 5,10-methylenTHF-Glu1; C: derivative of 5,10-methenylTHF-Glu1; D: derivative of THF-Glu1; E: derivative of DHF-Glu1; F: derivative of FA -Glu1.

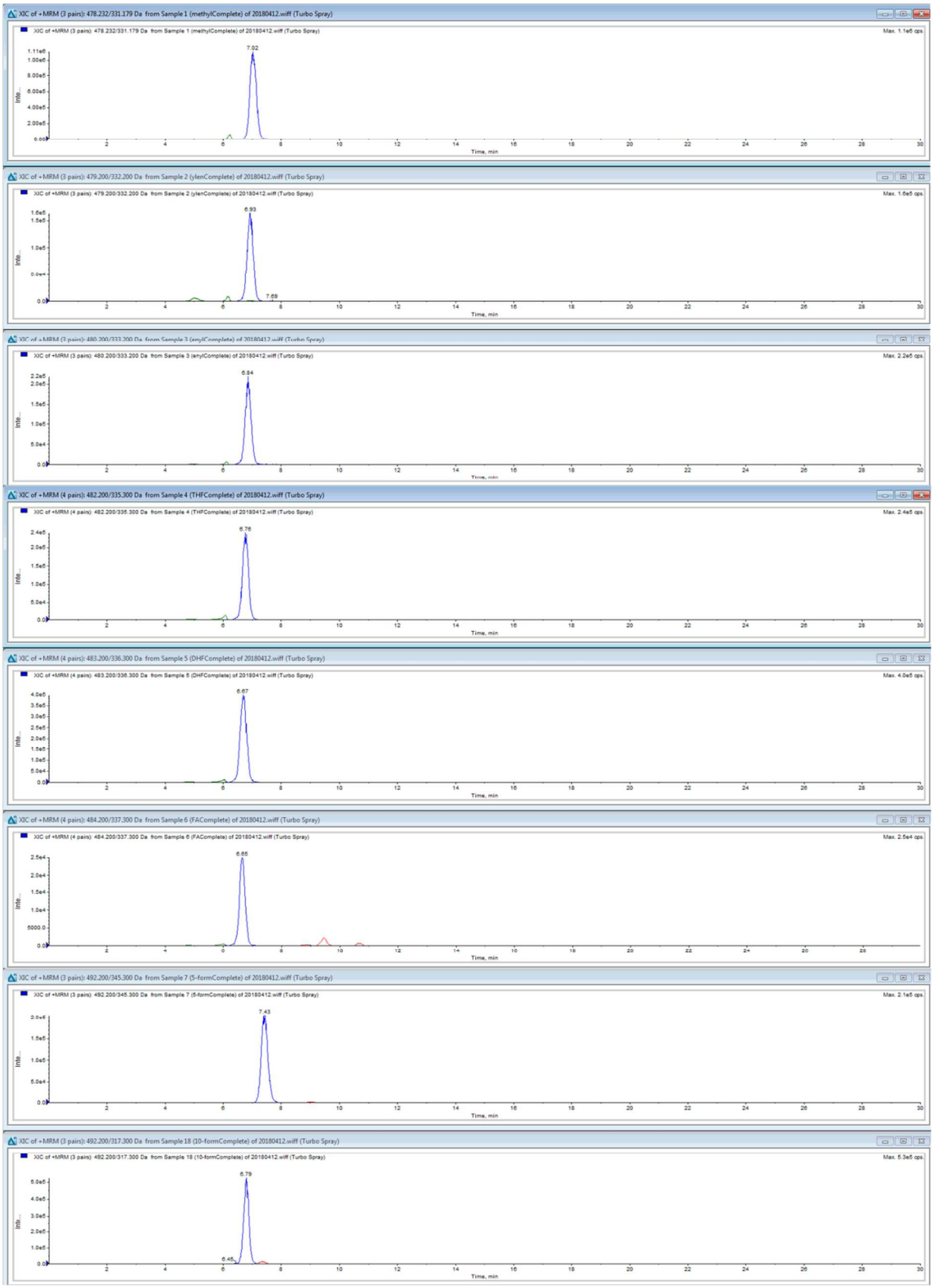

Fig. S3: Completeness and selectivity of derivatization reactions

Blue trace: expected product. Green trace: overmethylated compounds. Red trace: underivatized compound. Grey trace: undermethylated compound (where applicable). Top to bottom: 5-methylTHF; 5,10-methyleneTHF; 5,10-methenylTHF; THF; DHF; folic acid; 5-formylTHF, 10-formylTHF

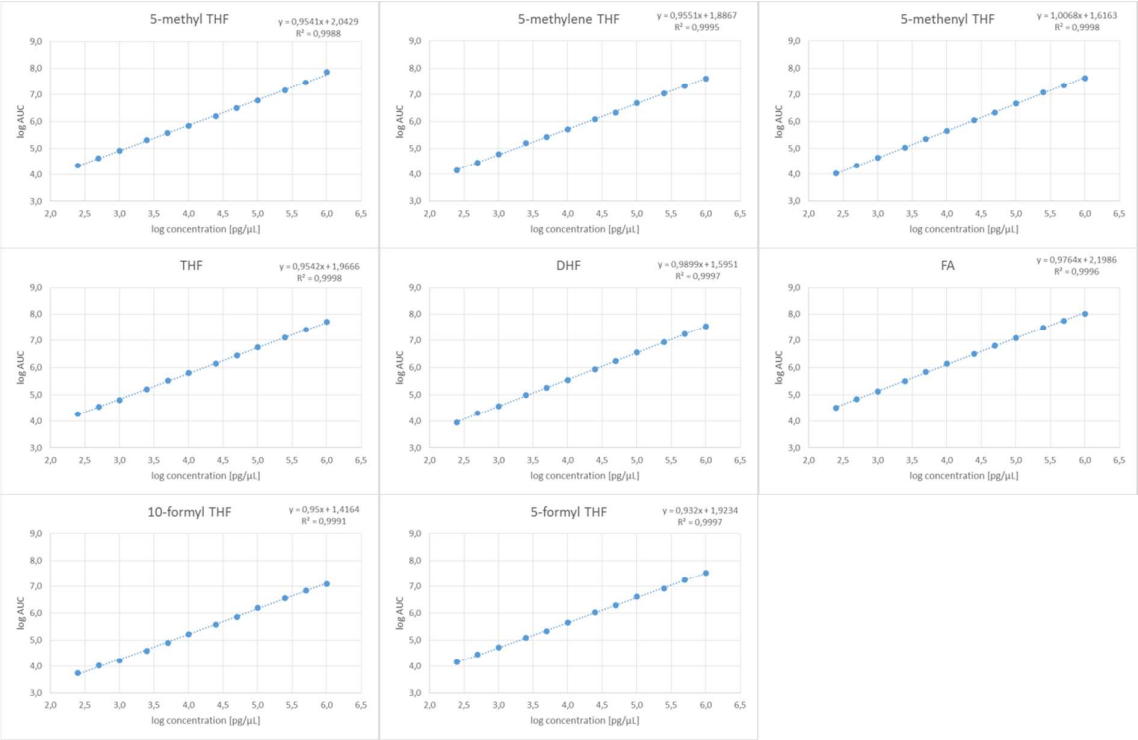

Fig. S4: Calibration of derivatized mono-glutamated folates. Both axes were weighted logarithmically.

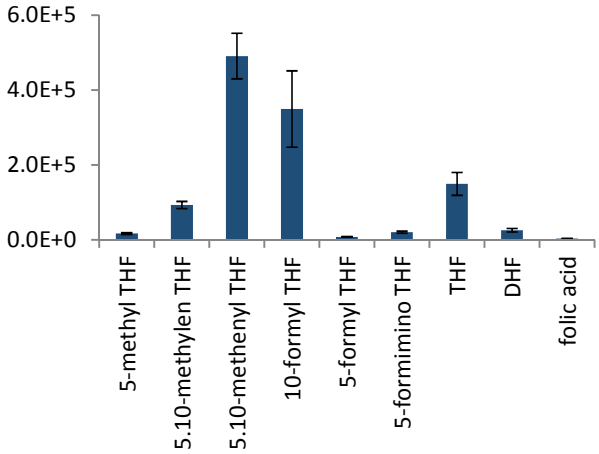

Fig. S5: Distribution of folate species in E. coli K12 grown on complex medium. Polyglutamation n=3.

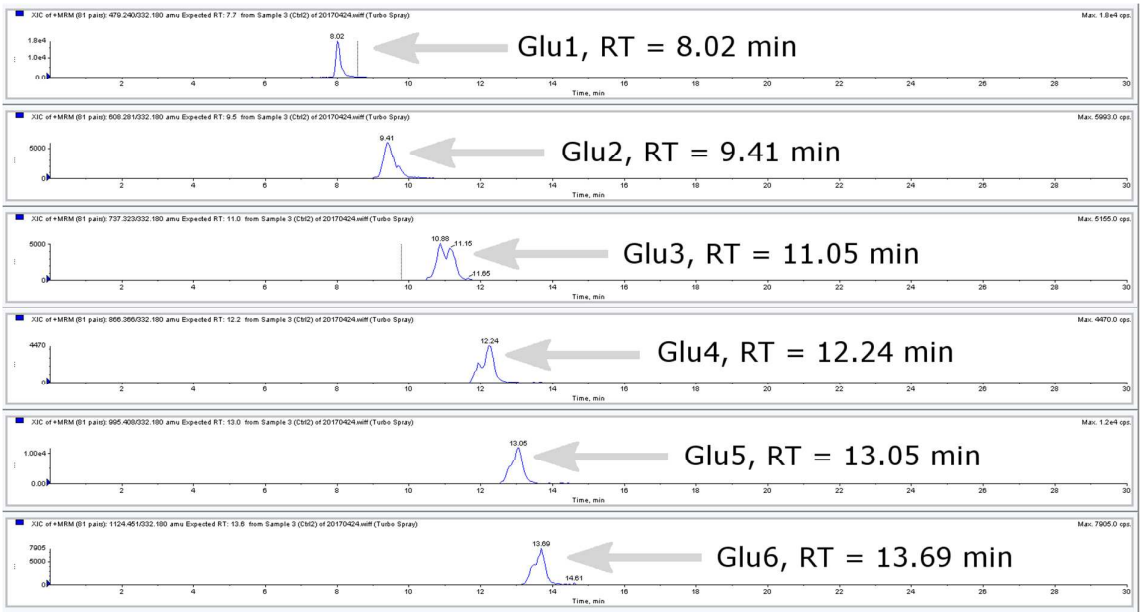

Fig. S6: Chromatographic separation of polyglutamation states. 5,10-methyleneTHF peaks of HepG2 cell extracts are shown with asccending polyglutamation number.

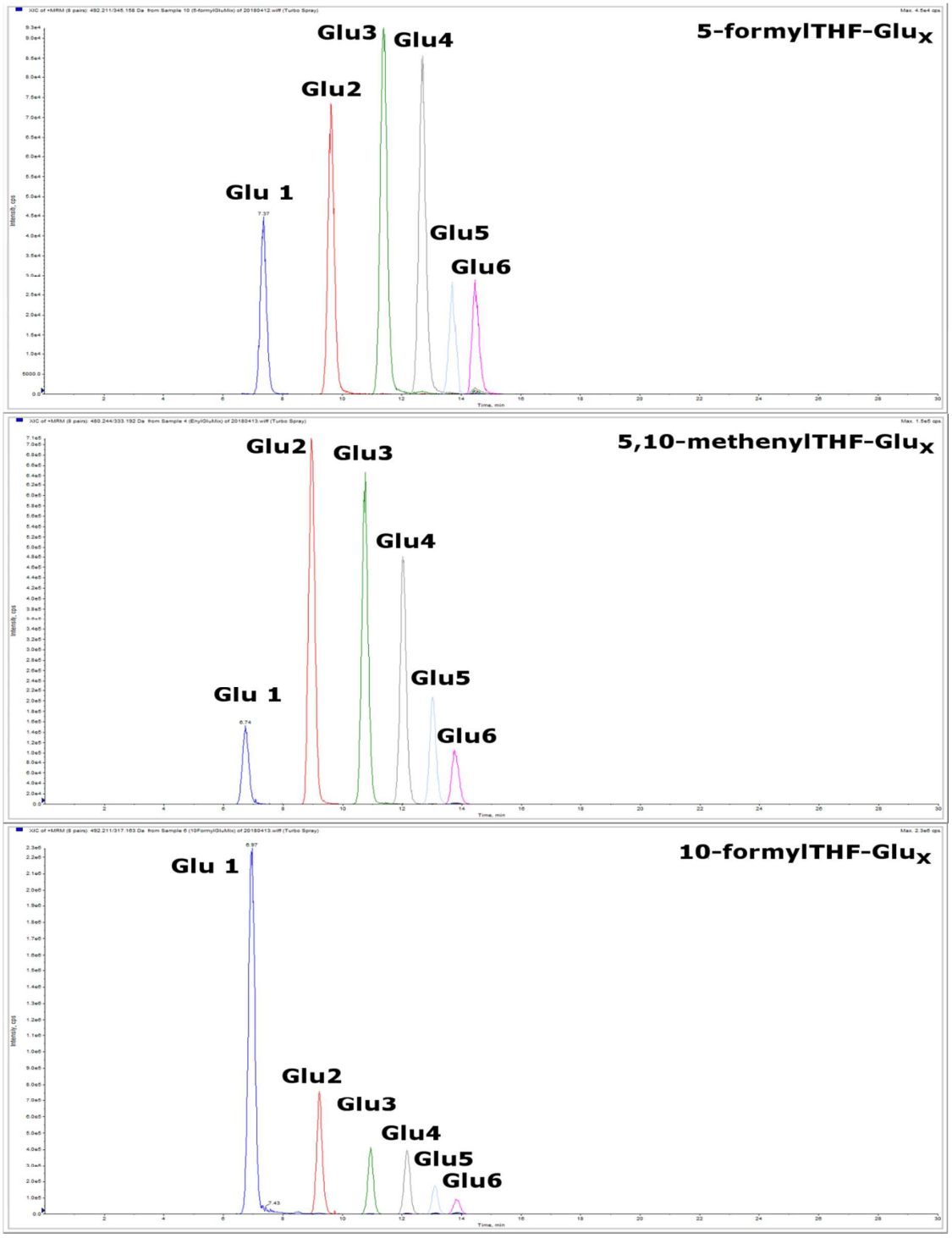

Fig. S7: Retention time standards of polyglutamation states. Top to bottom: derivatives of commercially available 5-formylTHF-Glu1 to Glu6; self-synthesized derivatives of 5,10-methenylTHF Glu1 to Glu6 (retention time standard for all 6 isotopologues); self-synthesized derivatives of 10-formylTHF-Glu1 to Glu6. 5,10-methenylTHF and 10-formylTHF standards were synthesized stepwise by pH shift from pH = 6.5 to pH = 1 (5,10-methenylTHF) and from pH = 1 to pH = 11 (10-formylTHF) and subsequent derivatization. Conversion was not complete in all cases and 10-formylTHF is prone to degradation before derivatization. Thus, self-synthesized standards were only used as retention time references.

Table S1: Analyte coverage of published methods. green tick: species covered individually by method verified by authentic standard; purple wave: species covered individually by method but no standard available; blue P: species measured as pool; red cross: not covered by method; 1) more than a single sample preparation and/or analytical run required; 2) it is unclear whether 5,10-methenylTHF was analysed separately as no transition is reported. Based on the 2013 paper by the same authors, the majority of 5,10-methenylTHF will convert to 5-formyl-THF during deconjugation under the conditions used. 10-formylTHF was measured in the pteridine ring oxidized forms 10-formylDHF and 10-formyl folic acid; 3) The authors reported interconversion of 5,10-methenylTHF to 5-formylTHF. To prevent this, they used a pH of 7.4. The 10-formyl species were measured in the ring oxidized form 10-formyl folic acid; 4) A pool of 5-formylTHF, 10-formylTHF and 5,10-methenylTHF was determined after acidification; 5) No measures to stabilize 5,10-methyleneTHF were reported.[1, 2, 3, 4, 5, 6, 7, 8]

	Schittmayer 2018	Chen 2017 **1)**	Ringling 2017 **2)**	Kiekens 2015 **3)**	Haandel 2012 **4)**	Kwon 2008 **5)**	Lu 2007 **5)**	Garratt 2005 **5)**
5-methylTHF	✓	✓	✓	✓	✓	✓	✓	✓
5,10-methylenTHF	✓	✓	✗	✗	✗	**5)**	**5)**	**5)**
5,10-methenylTHF	✓	P	✓ P	✓ P	P	✗	✓	✓
THF	✓	✓	✓	✓	✓	✓	✓	✓
DHF	✓	✓	✗	✗	✗	✓	✓	✓
folic acid	✓	✗	✓	✓	✗	✓	✓	✓
5-formylTHF	✓	✓	✓ P	✓ P	✓	P	P	P
10-formylTHF	✓	P	**2)**	**3)**	✗	P	P	P
5-formiminoTHF	∿	✗	✗	✗	✗	✗	✗	✗

Table S2: List of transitions for scheduled MRM method. Parents (Q1 Mass) indicated with * are doubly charged species. All analytes are representing their derivatives after reductive alkylation with labelled reagents.

Analyte	Q1 Mass (Da)	Q2 Mass (Da)	Time (min)	DP (volts)	CE (volts)
5-methylTHF-Glu1	478.232	331.179	7.0	110	35
5,10-methylen-Glu1	479.238	332.185	7.0	110	35
5,10-methenyl-Glu1	480.244	333.192	7.0	110	35
THF-Glu1	482.254	335.201	7.0	110	35
DHF-Glu1	483.260	336.208	7.0	110	35
FA-Glu1	484.267	337.214	7.0	110	35
5-formylTHF-Glu1	492.211	345.158	7.9	110	35
10-formylTHF-Glu1	492.211	317.163	7.3	110	35
5-formiminoTHF-Glu1	494.249	347.196	7.1	110	35
Int. Std. 5-methyl-THFGlu1	474.210	327.157	7.9	110	35
5-methylTHF-Glu2	607.274	331.179	9.4	110	44
5,10-methylen-Glu2	608.281	332.185	9.4	110	44
5,10-methenyl-Glu2	609.287	333.192	9.4	110	44
THF-Glu2	611.297	335.201	9.4	110	44
DHF-Glu2	612.303	336.208	9.4	110	44
FA-Glu2	613.309	337.214	9.4	110	44
5-formylTHF-Glu2	621.254	345.158	10.2	110	44
10-formylTHF-Glu2	621.254	317.163	9.8	110	44
5-formiminoTHF-Glu2	623.292	347.196	9.5	110	44
5-methylTHF-Glu3	736.317	331.179	11.1	110	54
5,10-methylen-Glu3	737.323	332.185	11.1	110	54
5,10-methenyl-Glu3	738.330	333.192	11.1	110	54
THF-Glu3	740.339	335.201	11.1	110	54
DHF-Glu3	741.345	336.208	11.1	110	54
FA-Glu3	742.352	337.214	11.1	110	54

5-formylTHF-Glu3	750.296	345.158	11.9	110	53
10-formylTHF-Glu3	750.296	317.163	11.4	110	53
5-formiminoTHF-Glu3	752.334	347.196	11.2	110	53
5-methylTHF-Glu4	865.360	331.179	12.3	110	63
5,10-methylen-Glu4	866.366	332.185	12.3	110	63
5,10-methenyl-Glu4	867.372	333.192	12.3	110	63
THF-Glu4	869.382	335.201	12.3	110	63
DHF-Glu4	870.388	336.208	12.3	110	63
FA-Glu4	871.394	337.214	12.3	110	63
5-formylTHF-Glu4	879.339	345.158	13.0	110	63
10-formylTHF-Glu4	879.339	317.163	12.5	110	63
5-formiminoTHF-Glu4	881.377	347.196	12.4	110	62
5-methylTHF-Glu5	994.402	331.179	13.1	110	73
5,10-methylen-Glu5	995.408	332.185	13.1	110	73
5,10-methenyl-Glu5	996.415	333.192	13.1	110	73
THF-Glu5	998.424	335.201	13.1	110	72
DHF-Glu5	999.431	336.208	13.1	110	72
FA-Glu5	1000.437	337.214	13.1	110	72
5-formylTHF-Glu5	1008.381	345.158	13.7	110	72
10-formylTHF-Glu5	1008.381	317.163	13.3	110	72
5-formiminoTHF-Glu5	1010.419	347.196	13.2	110	72
5-methylTHF-Glu6	1123.445	331.179	13.8	110	82
5,10-methylen-Glu6	1124.451	332.185	13.8	110	82
5,10-methenyl-Glu6	1125.457	333.192	13.8	110	82
THF-Glu6	1127.467	335.201	13.8	110	82
DHF-Glu6	1128.473	336.208	13.8	110	82
FA-Glu6	1129.479	337.214	13.8	110	82
5-formylTHF-Glu6	1137.424	345.158	14.4	110	81
10-formylTHF-Glu6	1137.424	317.163	14.0	110	81
5-formiminoTHF-Glu6	1139.462	347.196	13.9	110	81

5-methylTHF-Glu7	*627.251	331.179	14.4	110	70
5,10-methylen-Glu7	*627.754	332.185	14.4	110	70
5,10-methenyl-Glu7	*628.761	333.192	14.4	110	70
THF-Glu7	*634.758	335.201	14.4	110	70
DHF-Glu7	*635.763	336.208	14.4	110	70
FA-Glu7	*636.766	337.214	14.4	110	70
5-formylTHF-Glu7	*641.734	345.158	14.9	110	70
10-formylTHF-Glu7	*641.237	317.163	14.5	110	70
5-formiminoTHF-Glu7	*643.755	347.196	14.4	110	70
5-methylTHF-Glu8	*691.772	331.179	14.9	110	70
5,10-methylen-Glu8	*692.276	332.185	14.9	110	70
5,10-methenyl-Glu8	*693.282	333.192	14.9	110	70
THF-Glu8	*699.280	335.201	14.9	110	70
DHF-Glu8	*700.285	336.208	14.9	110	70
FA-Glu8	*701.288	337.214	14.9	110	70
5-formylTHF-Glu8	*706.256	345.158	15.4	110	70
10-formylTHF-Glu8	*705.758	317.163	15.0	110	70
5-formiminoTHF-Glu8	*708.277	347.196	14.9	110	70
glycine	112.115	66.110	6.5	50	30
serine	142.126	96.121	6.9	50	25
SAM	435.221	250.093	8.4	30	35
SAH	421.205	170.300	5.3	35	25
3-phospho-serine	222.092	176.087	9.5	30	30
TMP	323.064	80.973	7.5	30	30
IMP	349.054	137.046	10.1	60	20
AICAR	339.070	127.061	9.7	50	20
3-phospho-glycerate	187.000	98.980	11.4	30	25
NAD+	664.120	524.058	8.5	60	30
NADH	666.130	649.106	7.8	60	30
NADP+	744.083	604.025	11.4	60	30

NADPH	746.098	729.072	11.5	60	30
Sarcosine	108.093	62.088	6.6	50	30
N,N- dimethylglycine	104.071	58.066	6.6	50	30
Betaine	118.086	58.066	6.0	50	35
Choline	104.107	60.081	11.6	50	35
methionine	186.134	133.032	4.6	30	20
homocysteine	172.118	126.122	5.2	30	20
cysteine	158.103	58.995	5.7	60	30
glutathione	344.167	166.126	6.4	30	30
glutathione disulfide	685.311	166.126	9.4	30	45
cystathione	295.226	170.103	6.9	30	30

Bibliography supporting information

1. Chen, L.; Ducker, G. S.; Lu, W.; Teng, X.; Rabinowitz, J. D. *Anal. Bioanal. Chem.* **2017,** *409*, 5955-5964.
2. Ringling, C.; Rychlik, M. *Anal. Bioanal. Chem.* **2017,** *409*, 1815-1825.
3. Ringling, C.; Rychlik, M. *Eur. Food Res. Technol.* **2013,** *236*, 17-28.
4. Kiekens, F.; Van Daele, J.; Blancquaert, D.; Van Der Straeten, D.; Lambert, W. E.; Stove, C. P. *J. Chromatogr. A* **2015,** *1398*, 20-28.
5. Haandel, L.; Becker, M. L.; Williams, T.; Stobaugh, J.; Leeder, J. S. *Rapid Commun. Mass Spectrom.* **2012,** *26*, 1617-1630.
6. Kwon, Y. K.; Lu, W.; Melamud, E.; Khanam, N.; Bognar, A.; Rabinowitz, J. D. *Nat. Chem. Biol.* **2008,** *4*, 602-608.
7. Lu, W.; Kwon, Y. K.; Rabinowitz, J. D. *J. Am. Soc. Mass Spectrom.* **2007,** *18*, 898-909.
8. Garratt, L. C.; Ortori, C. A.; Tucker, G. A.; Sablitzky, F.; Bennett, M. J.; Barrett, D. A. *Rapid Commun. Mass Spectrom.* **2005,** *19*, 2390-2398.