Simple acid digestion procedure for the determination of total mercury in plankton by cold vapor atomic fluorescence spectroscopy

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Supplementary Material

Table S1. Description of digestion conditions applied at an initial phase to identify best conditions for the development and optimization of the procedure to obtain high recovery efficiency.

Condition	Nitric acid	Amount of nitric acid	Temperature	Time of digestion	Pre-Ultrasound
1*	50% v/v HNO_3	3 mL	85°C	12 hours	No
2*	65% HNO_3	3 mL	85°C	12 hours	No
3	50% v/v HNO_3	3 mL	85°C	12 hours	10 min.
4	65% HNO_3	3 mL	85°C	12 hours	10 min.
5	50% v/v HNO_3	3 mL	85°C	8 hours	10 min.
6	50% v/v HNO_3	3 mL	85°C	8 hours	No

*Conditions selected for the method optimization

Table S2. Digestion conditions and amounts of certified reference material IAEA-450 and BCR-414 and nitric acid (50% v/v HNO_3 or 65% w/w HNO_3) used on the first digestion batch.

CRM	Condition/Acid concentration	Replicate	Weight of CRM (g)	Weight of acid (g)
IAEA450	Condition 1 / 50% v/v HNO_3	R1	0.0032	3.71725
		R2	0.0022	3.79136
		R3	0.0038	3.76018
		R1	0.0196	3.81142
		R2	0.0204	3.78940
		R3	0.0185	3.69523
	Condition 2 / 65% w/w HNO_3	R1	0.0028	4.25916
		R2	0.0025	4.12656
		R3	0.0025	4.21956
		R1	0.0213	4.15093
		R2	0.0201	4.28112
		R3	0.0279	4.08583
BCR414	Condition 1 / 50% v/v HNO_3	R1	0.0028	3.78277
		R2	0.0035	3.79261
		R3	0.0020	3.75466
		R1	0.0204	3.75476
		R2	0.0189	3.78158
		R3	0.0254	3.79087
	Condition 2 / 65% w/w HNO_3	R1	0.0033	4.19161
		R2	0.0040	4.12320
		R3	0.0049	4.07007
		R1	0.0273	4.14747
		R2	0.0178	4.20749
		R3	0.0212	4.24887

Table S3. Digestion conditions and amounts of certified reference material IAEA-450 and BCR-414 and nitric acid (50% v/v HNO_3 or 65% w/w HNO_3) used on the second digestion batch.

CRM	Condition/Acid concentration	Replicate	Weight of CRM (g)	Weight of acid (g)
IAEA450	Condition 1 / 50% v/v HNO_3	R1	0.0042	3.75038
		R2	0.0024	3.73934
		R3	0.0034	3.48495
		R1	0.0242	3.69380
		R2	0.0261	3.60498
		R3	0.0273	3.78008
	Condition 2 / 65% w/w HNO_3	R1	0.0031	4.18116
		R2	0.0060	4.04270
		R3	0.0046	4.15616
		R1	0.0229	4.18374
		R2	0.0237	4.15771
		R3	0.0242	4.21530
BCR414	Condition 1 / 50% v/v HNO_3	R1	0.0035	3.42441
		R2	0.0048	3.75815
		R3	0.0053	3.73949
		R1	0.0287	3.76293
		R2	0.0220	3.73817
		R3	0.0212	3.67177
	Condition 2 / 65% w/w HNO_3	R1	0.0031	4.07674
		R2	0.0037	4.14168
		R3	0.0047	4.08100
		R1	0.0216	4.23301
		R2	0.0275	4.21778
		R3	0.0255	4.14943

Table S4. Digestion conditions and of the amounts of certified reference material IAEA-450 and BCR-414 and nitric acid (50% v/v HNO_3 or 65% w/w HNO_3) used on the third digestion batch. Na refers to any sample that did not pass the acceptance criteria of the analytical procedure.

CRM	Condition/Acid concentration	Replicate	Weight of CRM (g)	Weight of acid (g)
IAEA450	Condition 1 / 50% v/v HNO_3	R1	0.0233	3.77754
		R2	0.0212	3.76495
		R3	0.0219	3.76947
	Condition 2 / 65% w/w HNO_3	R1	0.0022	4.09883
		R2	0.003	4.17654
		R3	NA	NA
BCR414	Condition 2 / 65% w/w HNO_3	R1	0.0054	3.98134
		R2	0.003	3.89726
		R3	0.0029	4.2136
		R1	0.0214	4.21056
		R2	0.0279	4.06167
		R3	0.0238	4.07188

Table S5. Digestion conditions and amounts of certified reference material IAEA-450 and BCR-414 and nitric acid (50% v/v HNO_3 or 65% w/w HNO_3) used on the fourth digestion batch.

CRM	Condition/Acid concentration	Replicate	Weight of CRM (g)	Weight of acid (g)
IAEA450	Condition 1 / 50% v/v HNO_3	R1	0.0025	3.79083
		R2	0.0022	3.80043
		R3	0.0027	3.83353
		R1	0.0215	3.24528
		R2	0.0254	3.5572
		R3	0.0220	3.47809
	Condition 2 / 65% w/w HNO_3	R1	0.0024	4.14458
		R2	0.0023	4.11394
		R3	0.0028	3.39231
		R1	0.0247	4.19323
		R2	0.0213	4.21024
		R3	0.0196	4.21588
BCR414	Condition 1 / 50% v/v HNO_3	R1	0.0027	3.79581
		R2	0.0024	3.54409
		R3	0.0021	3.66467
		R1	0.0272	3.59568
		R2	0.0192	3.55636
		R3	0.0198	3.29032

Table S6. Results of digestion batch number 1 over 4. Information about CRM mass in each condition and its respective certified and measured concentrations. Additionally, accuracy, precision and recovery percentages are displayed.

CRM	Condition / Acid concentration	CRM mass [a] (g)			Certified content [a] (pg)			Measured content [a] (pg)			Accuracy Bias (%)	Recovery [a] (%)			Precision RSD [a] (%)
IAEA-450	Condition 1 /	0.0031	±	0.0008	318.9	±	84.1	278.5	±	87.2	-12.7%	86.4%	±	6.0%	6.9%
	50% v/v HNO_3	0.0195	±	0.0010	2028.0	±	99.2	1839.5	±	125.2	-9.3%	90.9%	±	9.2%	10.2%
	Condition 2 /	0.0026	±	0.0002	270.4	±	18.0	256.4	±	32.0	-5.2%	95.1%	±	13.4%	14.1%
	65% w/w HNO_3	0.0231	±	0.0042	2402.4	±	436.8	2413.1	±	745.0	0.4%	99.1%	±	12.2%	12.3%
BCR-414	Condition 1 /	0.0028	±	0.0008	763.6	±	207.2	686.7	±	170.9	-10.1%	90.3%	±	4.9%	5.4%
	50% v/v HNO_3	0.0216	±	0.0034	5952.4	±	939.3	5614.3	±	661.9	-5.7%	94.8%	±	8.0%	8.5%
	Condition 2 /	0.0041	±	0.0008	1122.4	±	221.4	1086.0	±	203.8	-3.2%	97.0%	±	7.1%	7.4%
	65% w/w HNO_3	0.0221	±	0.0048	6105.1	±	1321.1	5984.9	±	877.7	-2.0%	99.3%	±	11.1%	11.2%

[a] Mean ± SD (n=3): triplicate analysis of each Condition and CRM mass.

Table S7. Results of digestion batch number 2 over 4. Information about CRM mass in each condition and its respective certified and measured contents. Additionally, accuracy, precision and recovery percentages are displayed.

CRM	Condition / Acid concentration	CRM mass [a] (g)	Certified content [a] (pg)	Measured content [a] (pg)	Accuracy Bias (%)	Recovery [a] (%)	Precision RSD [a] (%)
IAEA-450	Condition 1 / 50% v/v HNO_3	0.0033 ± 0.0009	346.7 ± 93.8	299.9 ± 80.8	-13.5%	86.5% ± 6.9%	7.9%
		0.0259 ± 0.0016	2690.1 ± 162.6	2830.5 ± 408.1	-6.0%	93.9% ± 4.1%	4.4%
	Condition 2 / 65% w/w HNO_3	0.0046 ± 0.0015	474.9 ± 150.8	420.0 ± 129.1	-11.6%	88.7% ± 1.3%	1.5%
		0.0236 ± 0.0007	2454.4 ± 68.2	2404.5 ± 143.4	-2.0%	97.9% ± 3.2%	3.3%
BCR-414	Condition 1 / 50% v/v HNO_3	0.0045 ± 0.0009	1251.2 ± 256.4	1150.7 ± 244.5	-8.0%	91.9% ± 0.9%	1.0%
		0.0240 ± 0.0041	6614.8 ± 1136.7	5866.1 ± 1029.9	-11.3%	88.7% ± 1.9%	2.1%
	Condition 2 / 65% w/w HNO_3	0.0038 ± 0.0008	1058.0 ± 223.1	1018.6 ± 191.1	-3.7%	96.6% ± 2.2%	2.3%
		0.0249 ± 0.0030	6863.2 ± 828.2	6464.8 ± 832.6	-5.8%	94.1% ± 0.8%	0.9%

[a] Mean ± SD (n=3): triplicate analysis of each Condition and CRM mass.

Table S8. Results of digestion batch number 3 over 4. Information about CRM mass in each condition and its respective certified and measured concentrations. Additionally, accuracy, precision and recovery percentages are displayed.

CRM	Condition / Acid concentration	CRM mass [a] (g)	Certified content [a] (pg)	Measured content [a] (pg)	Accuracy Bias (%)	Recovery [a] (%)	Precision RSD (%)
IAEA-450	Condition 1 / 50% v/v HNO_3	0.0221 ± 0.0011	2301.9 ± 111.2	2182.9 ± 116.0	-5.2%	94.9% ± 6.1%	6.4%
	Condition 2 / 65% w/w HNO_3	0.0030 ± 0.0008	312.0 ± 58.8	713.6 ± 40.8	-6.4%	94.2% ± 5.4%	5.8%
BCR-414	Condition 2 / 65% w/w HNO_3	0.0038 ± 0.0014	1039.6 ± 390.6	1021.7 ± 388.5	-1.7%	98.2% ± 2.5%	2.6%
		0.0244 ± 0.0033	6725.2 ± 907.2	6339.5 ± 735.7	-5.7%	94.5% ± 4.2%	4.5%

[a] Mean ± SD (n=3): triplicate analysis of each Condition and CRM mass.

Table S9. Digestion batch number 4 over 4. Information about CRM mass in each condition and its respective certified and measured concentrations. Additionally, accuracy, precision and recovery percentages are displayed.

CRM	Condition / Acid concentration	CRM mass [a] (g)			Certified content [a] (pg)			Measured content [a] (pg)			Accuracy Bias (%)	Recovery [a] (%)			Precision RSD [a] (%)
IAEA-450	Condition 1 / 50% v/v HNO_3	0.0025	±	0.0003	256.5	±	26.2	221.5	±	28.7	-13.6%	86.3%	±	4.7%	5.5%
		0.0230	±	0.0021	2388.5	±	220.7	2184.6	±	233.0	-19.9%	91.4%	±	1.6%	1.8%
	Condition 2 / 65% w/w HNO_3	0.0025	±	0.0003	260.0	±	27.5	256.0	±	29.2	-1.5%	98.5%	±	4.7%	4.7%
		0.0219	±	0.0026	2274.1	±	270.1	2093.8	±	229.9	-7.9%	94.3%	±	4.5%	4.7%
BCR-414	Condition 1 / 50% v/v HNO_3	0.0024	±	0.0003	665.2	±	78.7	616.9	±	72.4	-7.3%	92.8%	±	3.2%	3.4%
		0.0221	±	0.0045	6090.4	±	1229.8	5211.6	±	962.0	-14.4%	85.8%	±	1.4%	1.6%

[a] Mean ± SD (n=3): triplicate analysis of each Condition and CRM mass.

Table S10. Estimation of the minimum amount of total mercury necessary to be present in 2 mg and 20 mg sample considered to the LOQ generated from the different conditions and the dilution (three times) performed for the analysis in triplicate. Results are expressed in ng of mercury per gram of sample.

Amount of total Hg required per sample (ng g^{-1})			
Condition 1 /50% v/v HNO3		**Condition 2 /65% w/w HNO3**	
LOQ: 0.0049 ng g^{-1}		LOQ: 0.0107 ng^{-1}	
2 mg of sample	20 mg of sample	2 mg of sample	20 mg of sample
7.4	0.7	16.0	1.6

Table S11. Total mercury concentration of planktonic sample from Leman Lake (n=2).

Planktonic sample from Leman Lake (pg Hg mg^{-1} dry weight)			
Replicates	**Average**	**SD**	**RSD**
10.6 9.7	10.1	0.6	6%

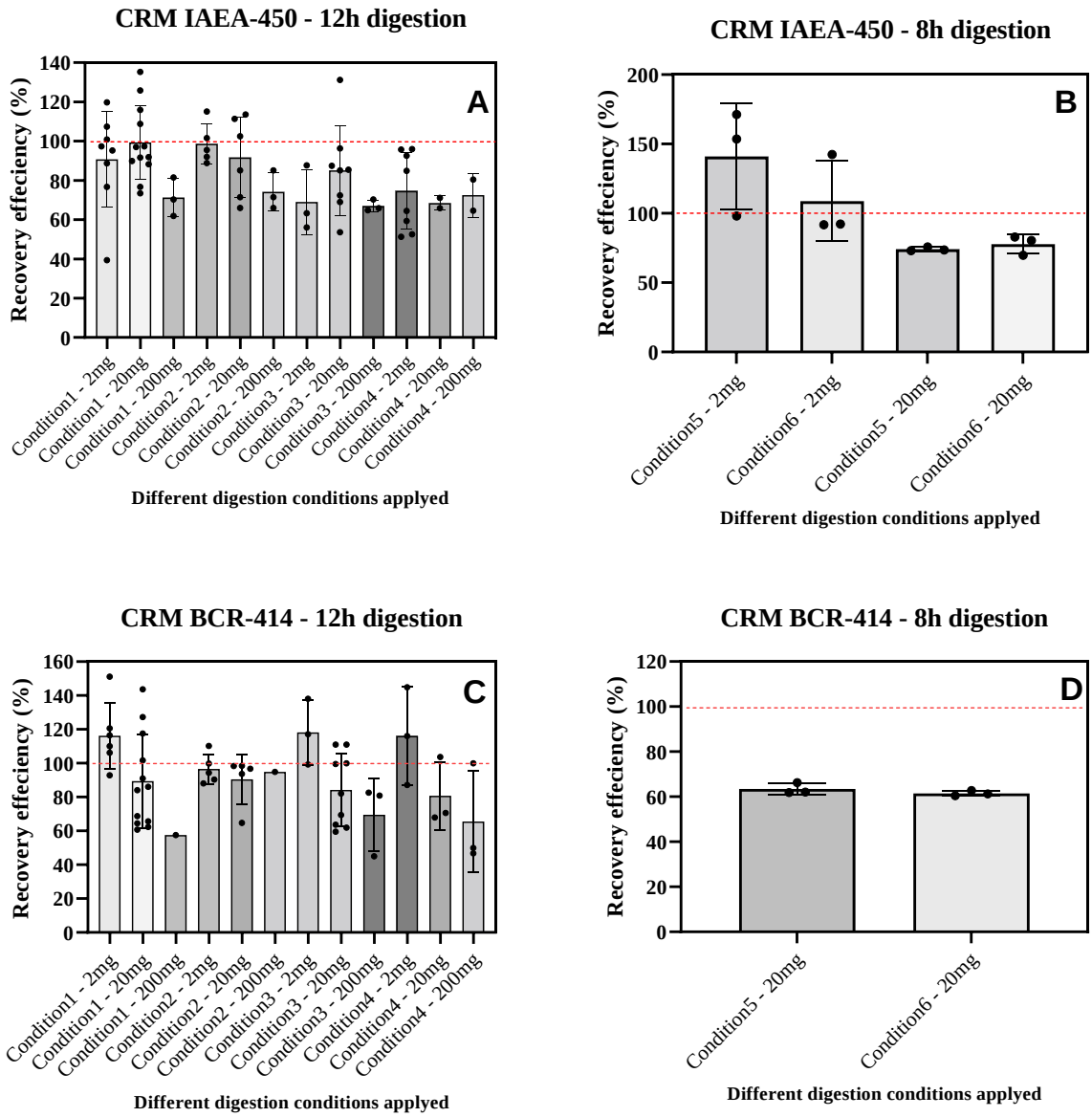

Figure S1. Recovery efficiency results from the initial screening performed with different digestion conditions (Condition 1 and 6: 50% v/v HNO_3 without ultrasound (WTU); Condition 2: 65% HNO_3 WTU; Condition 3 and 5: 50% v/v HNO_3 with ultrasound (WU); Condition 4: 65% HNO_3 WU). More details about the different conditions are described in Table S1. Different dots correspond to different replicates measured. Digestion and replicates were performed along different runs.

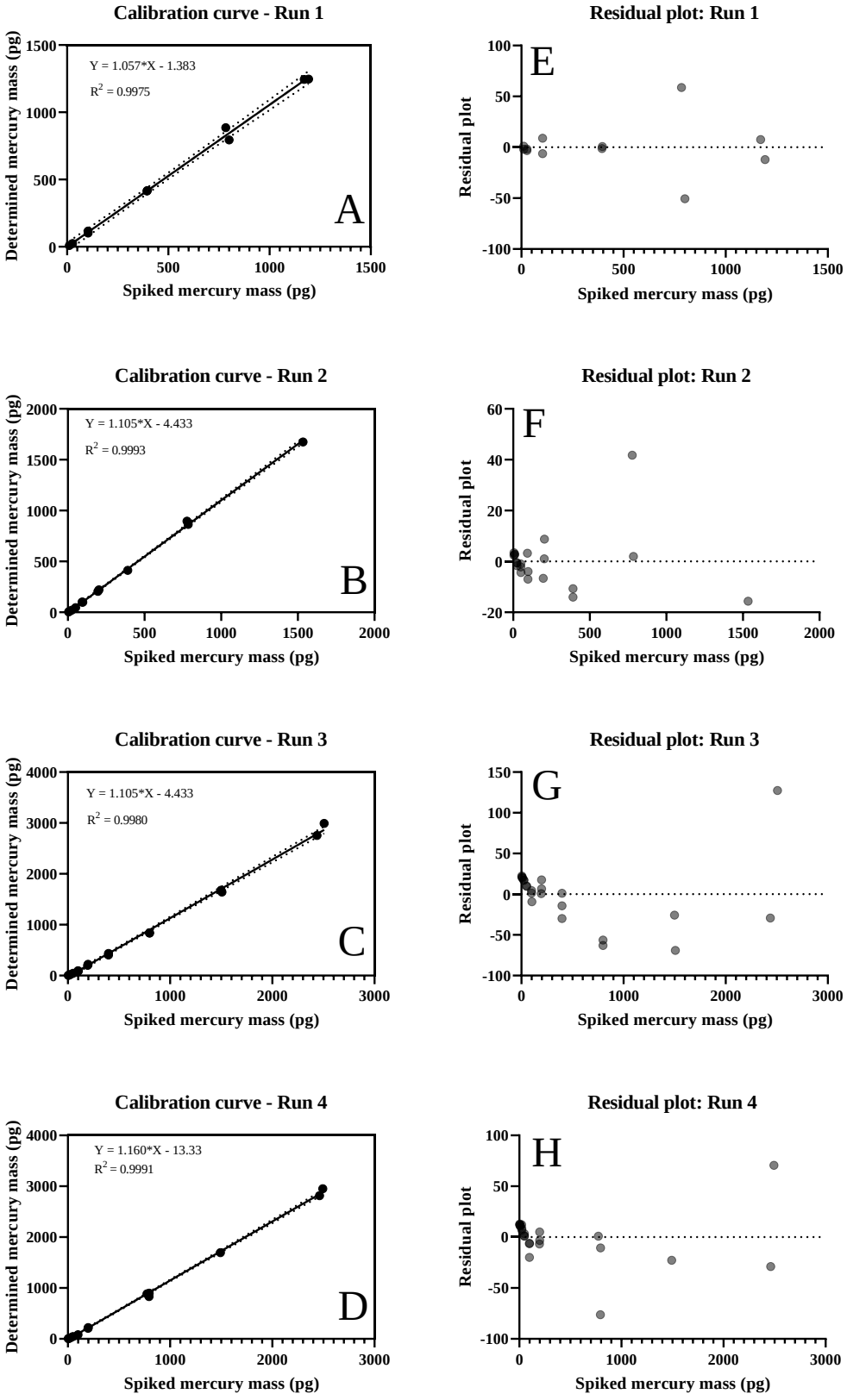

Figure S2. Calibration and residual plots of each run analysis.