SUPPORTING INFORMATION

Hg isotope ratio measurements of methylmercury in fish tissues using HPLC with off line cold vapour generation MC-ICPMS

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Table S1. Relative Hg isotope ratio data obtained by HPLC-CVG-MC-ICPMS from repeated measurements of CH_3Hg in BCR 463 reference material relative to NIST SRM 3133 standard. Concentration of Hg in measured solutions ranged from 10 to 15 ng g^{-1}. Uncertainty terms are standard deviation of individual measurement at 70% confidence level.

Sample	Methylmercury							
	$\delta^{199/198}Hg$	STD, ‰	$\delta^{200/198}Hg$	STD, ‰	$\delta^{201/198}Hg$	STD, ‰	$\delta^{202/198}Hg$	STD, ‰
1	1.97	0.14	0.49	0.10	1.99	0.12	0.61	0.12
2	1.94	0.08	0.41	0.07	1.98	0.09	0.64	0.13
3	2.02	0.06	0.35	0.08	1.86	0.10	0.65	0.12
4	1.92	0.07	0.46	0.06	1.92	0.11	0.74	0.10
5	2.15	0.10	0.43	0.09	2.30	0.10	0.52	0.13
6	2.05	0.13	0.42	0.11	2.11	0.12	0.66	0.12
7	2.03	0.22	0.41	0.14	2.03	0.14	0.71	0.18
8	2.13	0.20	0.49	0.12	2.31	0.12	0.58	0.18
9	2.19	0.17	0.49	0.12	2.46	0.09	0.73	0.15
10	1.95	0.17	0.31	0.16	1.98	0.19	0.54	0.18
11	1.90	0.14	0.33	0.13	1.76	0.18	0.56	0.15
12	2.08	0.15	0.41	0.11	2.20	0.11	0.54	0.18
13	1.94	0.21	0.29	0.14	1.88	0.15	0.47	0.16
14	1.91	0.12	0.23	0.26	1.77	0.18	0.56	0.10
15	2.10	0.12	0.33	0.11	2.30	0.06	0.59	0.13
16	2.04	0.21	0.38	0.13	2.17	0.10	0.62	0.20

Table S2. Relative Hg isotope ratio data obtained by CVG-MC-ICPMS from repeated measurements of bulk Hg in BCR 463 reference material relative to NIST SRM 3133 standard. Concentration of Hg in measured solutions ranged from 10 to 20 ng g^{-1}. Uncertainty terms are standard deviation of individual measurement at 70% confidence level.

Sample	Bulk Hg							
	$\delta^{199/198}Hg$	STD, ‰	$\delta^{200/198}Hg$	STD, ‰	$\delta^{201/198}Hg$	STD, ‰	$\delta^{202/198}Hg$	STD, ‰
1	1.91	0.11	0.45	0.13	2.16	0.08	0.71	0.17
2	1.85	0.14	0.44	0.13	1.93	0.19	0.63	0.14
3	1.85	0.18	0.44	0.16	1.91	0.15	0.75	0.16
4	1.98	0.07	0.36	0.06	2.20	0.07	0.67	0.08
5	1.94	0.08	0.36	0.07	2.19	0.09	0.59	0.10
6	1.95	0.07	0.42	0.06	2.30	0.06	0.74	0.10
7	1.97	0.06	0.43	0.06	2.38	0.06	0.58	0.08
8	2.11	0.14	0.48	0.12	2.10	0.13	0.79	0.18
9	1.98	0.13	0.46	0.09	2.13	0.09	0.69	0.13

Table S3. Relative Hg isotope ratio data obtained by HPLC-CVG-MC-ICPMS from repeated measurements of methylmercury in NIST SRM 1947 reference material relative to NIST SRM 3133 standard. Concentration of Hg in measured solutions was 10 ng g^{-1}. Uncertainty terms are standard deviation of individual measurement at 70% confidence level.

Sample	Methylmercury							
	$\delta^{199/198}$Hg	STD, ‰	$\delta^{200/198}$Hg	STD, ‰	$\delta^{201/198}$Hg	STD, ‰	$\delta^{202/198}$Hg	STD, ‰
1	5.15	0.26	0.59	0.21	4.89	0.15	0.98	0.24
2	5.20	0.25	0.55	0.20	4.90	0.22	1.05	0.27
3	5.59	0.21	0.54	0.23	4.92	0.22	1.00	0.21
4	5.43	0.25	0.58	0.16	4.56	0.23	1.05	0.23
5	5.39	0.20	0.58	0.13	5.13	0.15	1.13	0.13
6	5.44	0.22	0.55	0.23	4.81	0.24	1.03	0.29
7	5.62	0.25	0.57	0.16	4.89	0.20	1.07	0.18
8	5.56	0.16	0.62	0.16	4.95	0.21	1.12	0.18
9	5.73	0.19	0.55	0.21	5.09	0.24	1.02	0.24
10	5.48	0.16	0.60	0.14	4.96	0.18	1.08	0.22

Table S4. Relative Hg isotope ratio data obtained by CVG-MC-ICPMS from repeated measurements of bulk Hg in NIST SRM 1947 reference material relative to NIST SRM 3133 standard. Concentration of Hg in measured solutions was 10 ng g^{-1}. Uncertainty terms are standard deviation of individual measurement at 70% confidence level.

Sample	Bulk Hg							
	$\delta^{199/198}$Hg	STD, ‰	$\delta^{200/198}$Hg	STD, ‰	$\delta^{201/198}$Hg	STD, ‰	$\delta^{202/198}$Hg	STD, ‰
1	5.35	0.13	0.55	0.08	4.96	0.14	1.11	0.13
2	5.46	0.12	0.59	0.13	5.30	0.20	1.28	0.15
3	5.62	0.20	0.62	0.21	5.12	0.24	1.15	0.21
4	5.51	0.22	0.68	0.17	5.23	0.17	1.16	0.14
5	5.35	0.23	0.54	0.19	4.98	0.21	1.38	0.17
6	5.51	0.19	0.60	0.15	5.09	0.17	1.28	0.21

Table S5. Instrumental operating conditions employed in the HPLC procedure

Chromatography	
Column	PROTECOL C18G123 PC3 3 µm 120 Å; 100 mm × 10 mm id
Column oven temperature	20 °C
Eluent	0.05% m m^{-1} L-cysteine, 0.006% v m^{-1} acetic acid adjusted to pH 4 with ammonia
Mode	Ion pair
Flow rate	0.7 mL min^{-1}
Sample injection volume	0.5 mL
Volume of HPLC fractions collected	3 mL

Experimental set-up used in online coupling of HPLC to CVG-MC-ICPMS

The instrumental configuration we used is shown in Fig. S1. A mobile phase mobile phase containing 0.05% m m^{-1} L-cysteine / 0.006% v m^{-1} acetic acid at pH 4 was pumped at a flow rate ranging from 0.1 to 1 mL min^{-1} through a reverse phase C18 column (PROTECOL C18G123 100 x 10 mm) from Kinesis Ltd (St Neots, Cambridgeshire, UK). Post-column dissociation of CH$_3$Hg to inorganic Hg was achieved by UV irradiation and addition of strong oxidising substances via T-piece connection. The oxidising substances included 2% BrCl solution with addition of Fe and H$_2$O$_2$ at concentrations of 100 µg g^{-1} and 2%, respectively, to initiate the Fenton type reaction. The flow rates of HPLC mobile phase and solution

containing the oxidative agents were matched at 1:1 ratio. 5% SnCl$_2$ solution was used as a reductant in CVG chamber.
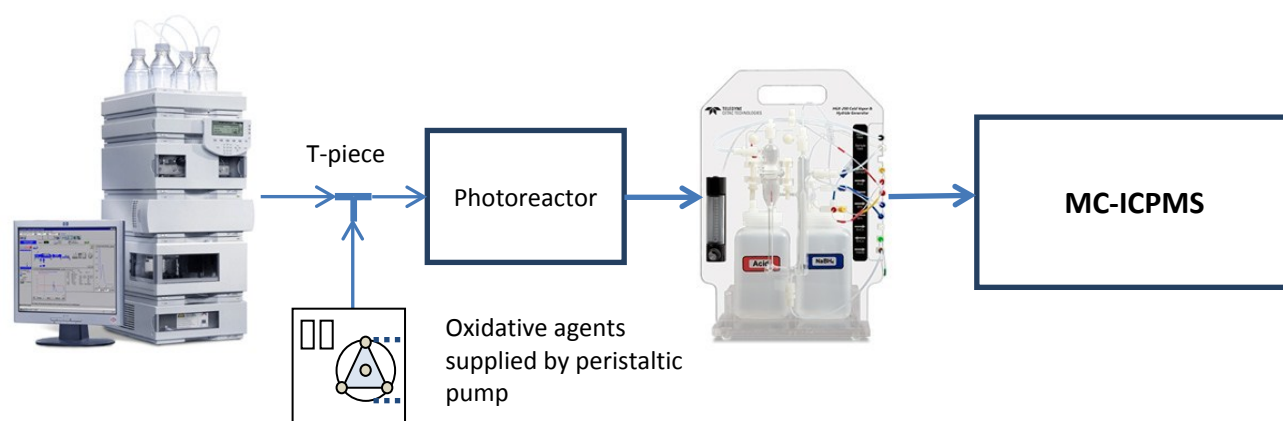

Fig. S1. Schematic illustration of the instrumental set-up used in online coupling of HPLC to CVG MC-ICPMS.

Performance of online Hg isotope ratio measurements in CH$_3$Hg fraction

Generation of cold Hg vapour from CH$_3$Hg using SnCl$_2$ as a reductant requires an extra step in the measurement procedure to convert CH$_3$Hg into iHg. This conversion can be performed by breaking down organo-Hg species using UV light, strong oxidising agent or combined action of both.[26,27,43] Attempts to perform Hg isotope ratio measurements of CH$_3$Hg by on-line coupling of the developed chromatographic procedure to CVG MC-ICPMS using the aforementioned approaches were unsuccessful. The results of isotopic measurements were erratic.

The main source of bias in measured isotope ratios was found to be the inability to achieve quantitative conversion of CH$_3$Hg into iHg prior to entering CVG reaction chamber using the developed procedure. It is known that dissociation of CH$_3$Hg can be accompanied by both mass-dependent and mass-independent Hg isotope fractionation.[4,5] As a consequence, non-quantitative conversion of CH$_3$Hg into iHg leads to artificial Hg isotope fractionation. In addition, matrix effect due to the presence of reduced sulphur in the mobile phase of HPLC contributed to bias in the results.

The fact that rapid quantitative dissociation of CH$_3$Hg in the mobile phase is a significant analytical challenge is well illustrated by the experiments we performed in order to assess kinetics of the process. These involved exposure of CH$_3$Hg solution with the matrix identical to that of the HPLC mobile phase to

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UV light and an attack of oxidative radicals over controlled periods of time, followed by Hg isotopic analysis by CVG MC-ICPMS. As can be seen from Fig. 2, either approximately 30 min of exposure to combined action of UV light and oxidative radicals produced by Fenton-type reaction or a few hours of Fenton-type reaction in the dark are needed to complete conversion of CH_3Hg into iHg. Furthermore, the experimental data shows that the chemical composition of the solution plays a crucial role in the process. Photo-dissociation of CH_3Hg in dilute HNO_3 solution proceeds faster than that in a solution which mimics the mobile phase matrix (Fig. S2). A plausible explanation of this observation is that dissociation of methyl group donor compounds, such as acetic acid, results in releasing a methyl radical into solution. The reaction can be presented as follows[44]

$$CH_3COOH + h\nu \rightarrow CH_3^\bullet + {}^\bullet COOH \tag{6}$$

A methyl radical can then react with iHg to re-generate a molecule of CH_3Hg. Simultaneously occurring processes of break down and re-generation of CH_3Hg, both accompanied by Hg isotope fractionation, lead to erratic and poorly reproducible Hg isotope data.

The results of Hg isotope ratio measurement with online coupling of HPLC to CVG-MC-ICPMS show that hyphenating HPLC online to CVG-MC-ICPMS for determination of the isotopic composition of Hg species is a significantly more challenging task than using one for quantifying concentrations of Hg species. The key difference seems to lie in the level of precision required in the measurements. While 10% to 15% measurement uncertainty associated with quantifying mass fraction of CH_3Hg in a sample at environmentally relevant concentration level is fit for purpose, loss in CH_3Hg recovery of 10-15% due to its non-quantitative conversion into inorganic Hg and volatilisation from solution can result in considerable bias in Hg isotopic measurements. As also follows from the results, Hg isotopic measurements by online HPLC-CVG-MC-ICPMS could prove viable if HPLC procedure is significantly modified with the use of more efficient online conversion of CH_3Hg to inorganic Hg.
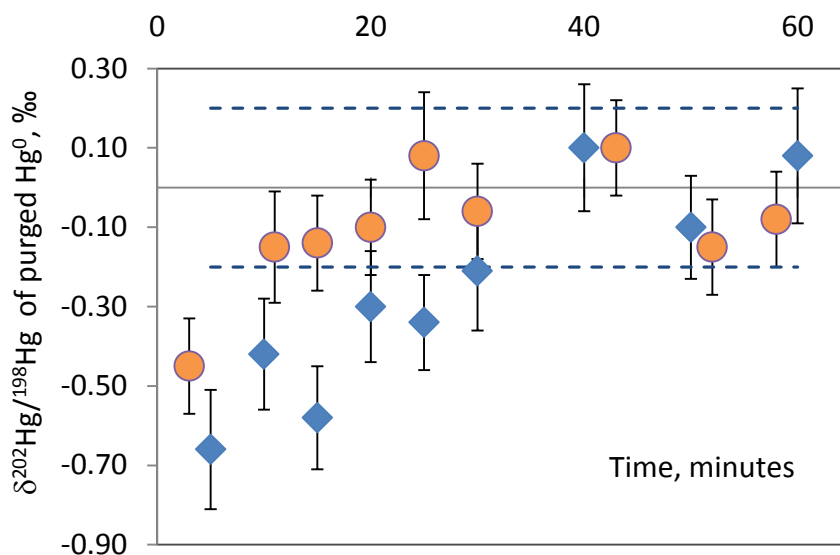
Fig. S2. $\delta^{202/198}$Hg values of CH$_3$Hg solutions plotted vs time elapsed since the onset of CH$_3$Hg dissociation reaction induced by UV irradiation and oxidative radicals produced by Fenton type reaction. The latter was initiated by adding Fe and H$_2$O$_2$ at concentrations of 100 μg g^{-1} and 2%, respectively. Circles represent the results obtained in sample matrix containing only 2% HNO$_3$. Diamonds represent the results obtained in sample matrix mimicking the mobile phase of HPLC (0.05% m m^{-1} L-cysteine / 0.006% v m^{-1} CH$_3$COOH). $\delta^{202/198}$Hg values of the solutions were determined by CVG MC-ICPMS relative to the stock solution of CH$_3$Hg, converted to the inorganic form by microwave assisted digestion. Hg concentration level in solutions was 20 ng g^{-1}. Uncertainty bars are standard deviation at 1 σ level. Dotted lines are the 95% confidence interval of the measurements.