

**ELECTRONIC SUPPORTING INFORMATION**

**COMPARISON OF GC-ICP-MS, GC-EI-MS AND GC-EI-MS/MS FOR THE DETERMINATION OF METHYLMERCURY, ETHYLMERCURY AND INORGANIC MERCURY IN BIOLOGICAL SAMPLES BY TRIPLE SPIKE SPECIES-SPECIFIC ISOTOPE DILUTION MASS SPECTROMETRY**

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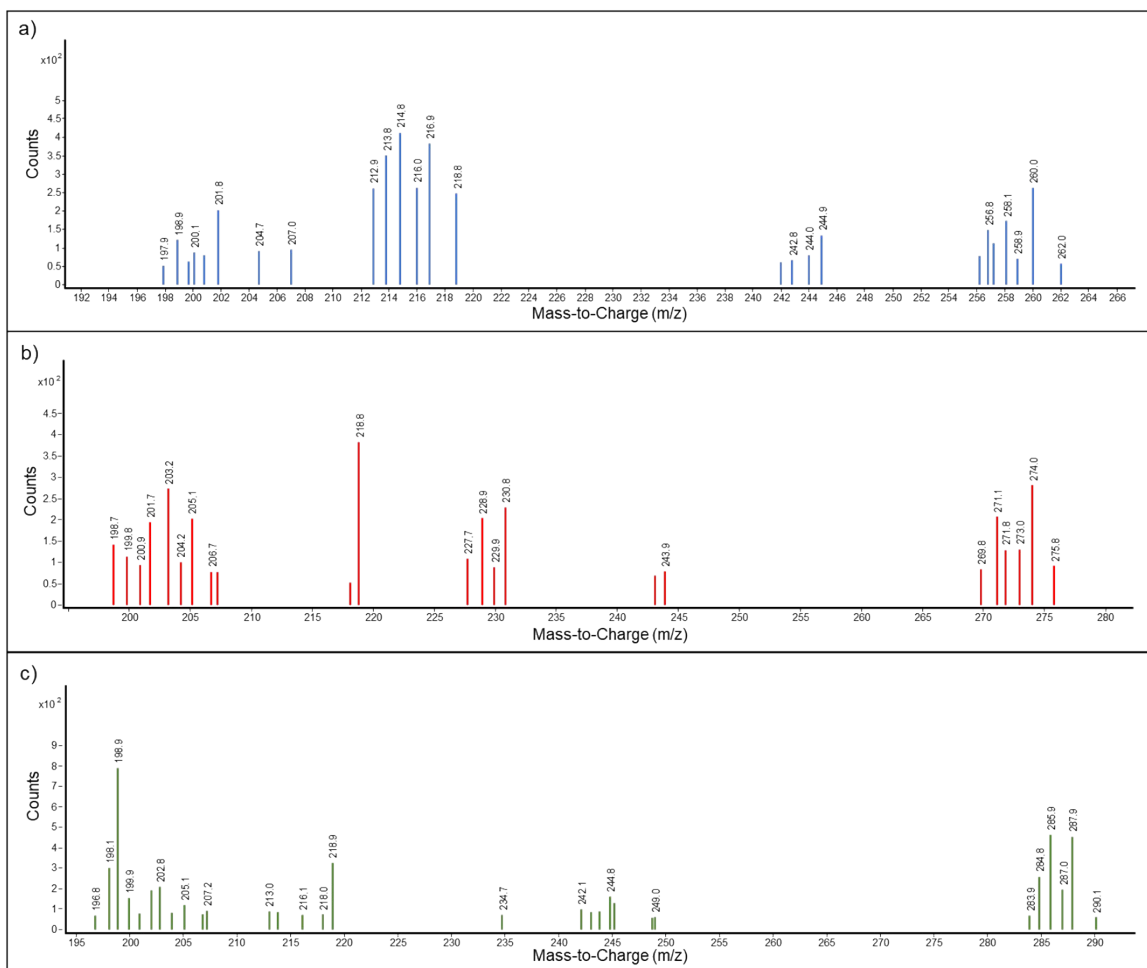
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**Figure S1.** Mass spectra of a natural abundance standard of the three propylated mercury compounds obtained by GC-MS in SCAN mode: a) MeHgPr<sup>+</sup>; b) EtHgPr<sup>+</sup>; c) HgPr<sub>2</sub><sup>+</sup>. The cluster for the molecular ion is observed at the right side of the mass spectra.



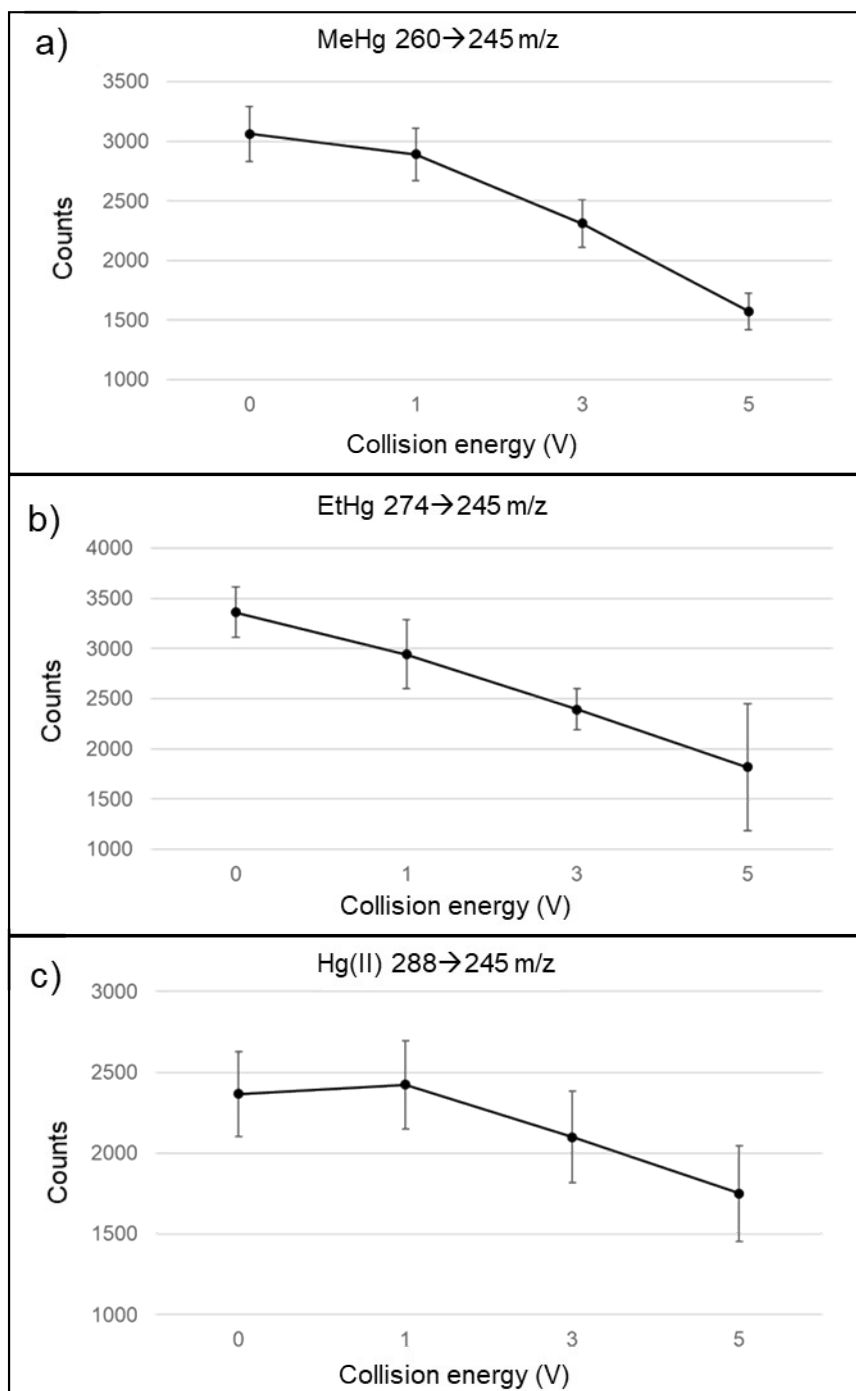
**Table S1.** Spectral purity of the molecular ion cluster for the three propylated mercury species. Contributions (%) of M, M-1 to the experimental spectra are calculated by multiple linear regression as described previously<sup>27</sup>. The uncertainty of the values corresponds to the standard deviations of n= 5 GC-EI-MS injections of a natural abundance standard.

	<b>M</b>	<b>M-1</b>
<b>MeHgPr<sup>+</sup></b>	98.55 ± 0.68	1.57 ± 0.69
<b>EtHgPr<sup>+</sup></b>	98.84 ± 0.55	1.25 ± 0.57
<b>HgPr<sup>2+</sup></b>	98.82 ± 0.37	1.30 ± 0.38

**Table S2.** Theoretical isotopic distributions of the molecular clusters for natural abundance and labelled propylated mercury compounds calculated by the algorithm proposed by Kubinyi<sup>25</sup>. The experimental isotopic distributions obtained by GC-ICP-MS for mercury in these compounds are also given for comparison.

	m/z	Natural	<sup>201</sup> MeHg	<sup>198</sup> EtHg	<sup>199</sup> Hg(II)
MePrHg <sup>+</sup>	256	0.095	0.001	0.922	0.013
	257	0.166	0.001	0.053	0.861
	258	0.228	0.010	0.007	0.083
	259	0.136	0.919	0.005	0.013
	260	0.291	0.066	0.007	0.022
EtPrHg <sup>+</sup>	270	0.094	0.001	0.912	0.013
	271	0.165	0.001	0.063	0.851
	272	0.228	0.010	0.007	0.092
	273	0.137	0.910	0.005	0.014
	274	0.290	0.075	0.007	0.021
Pr <sub>2</sub> Hg <sup>+</sup>	284	0.093	0.001	0.902	0.013
	285	0.164	0.001	0.072	0.842
	286	0.227	0.010	0.008	0.100
	287	0.138	0.900	0.005	0.015
	288	0.288	0.085	0.007	0.021
Hg <sup>+</sup> (ICP-MS)	198	0.100	0.001	0.982	0.013
	199	0.169	0.001	0.002	0.909
	200	0.231	0.010	0.004	0.042
	201	0.132	0.961	0.004	0.010
	202	0.299	0.026	0.005	0.019

**Figure S2.** Collision energy optimization from 0 to 5 V: a) 260 → 245 m/z MeHg transition; b) 274 → 245 m/z EtHg transition; c) 288 → 245 Hg(II) transition.



**Table S3.** Comparison of the theoretical isotopic distributions calculated as described by Ramaley *et al*<sup>26</sup> with the experimental distributions obtained by GC-MS/MS. The standard uncertainty of the results corresponds to n=5 independent GC-MS/MS injections of a natural abundance standard of the three compounds

	Precursor ion (m/z)	Product ion (m/z)	Theoretical isotopic distribution (%)	Experimental isotopic distribution (%)
MePrHg <sup>+</sup>	256	241	10.48	10.55 ± 0.03
	257	242	18.09	18.15 ± 0.11
	258	243	24.88	25.02 ± 0.03
	259	244	14.67	14.69 ± 0.04
	260	245	31.87	31.58 ± 0.10
EtPrHg <sup>+</sup>	270	241	10.48	10.61 ± 0.04
	271	242	18.09	18.37 ± 0.04
	272	243	24.88	24.95 ± 0.10
	273	244	14.67	14.57 ± 0.03
	274	245	31.87	31.87 ± 0.08
Pr <sub>2</sub> Hg <sup>+</sup>	284	241	10.48	10.66 ± 0.04
	285	242	18.09	18.41 ± 0.07
	286	243	24.88	24.96 ± 0.13
	287	244	14.67	14.56 ± 0.05
	288	245	31.87	31.41 ± 0.15

**Table S4.** Theoretical isotopic distributions of the selected SRM for natural and labelled mercury propylated compounds calculated as described by Ramaley et al<sup>26</sup>.

	SRM transition	Natural	<sup>201</sup> MeHg	<sup>198</sup> EtHg	<sup>199</sup> Hg(II)
<b>MePrHg<sup>+</sup> → PrHg<sup>+</sup></b>	<b>256→241</b>	0.106	0.000	0.954	0.011
	<b>257→242</b>	0.182	0.002	0.034	0.890
	<b>258→243</b>	0.250	0.009	0.003	0.070
	<b>259→244</b>	0.147	0.935	0.004	0.010
	<b>260→245</b>	0.316	0.054	0.005	0.019
<b>EtPrHg<sup>+</sup> → PrHg<sup>+</sup></b>	<b>270→241</b>	0.106	0.000	0.952	0.011
	<b>271→242</b>	0.184	0.001	0.034	0.890
	<b>272→243</b>	0.249	0.007	0.004	0.070
	<b>273→244</b>	0.146	0.940	0.004	0.010
	<b>274→245</b>	0.315	0.051	0.005	0.019
<b>Pr<sub>2</sub>Hg<sup>+</sup> → PrHg<sup>+</sup></b>	<b>284→241</b>	0,107	0,000	0,954	0,012
	<b>285→242</b>	0,184	0,001	0,034	0,889
	<b>286→243</b>	0,250	0,007	0,003	0,070
	<b>287→244</b>	0,146	0,940	0,004	0,011
	<b>288→245</b>	0,314	0,051	0,005	0,018

### Comparison of detection and quantification limits

Due to the absence of samples free of mercury species, the blank values were measured by triple spiking IDMS adding proper amounts of <sup>201</sup>Hg-enriched MeHg, <sup>199</sup>Hg-enriched Hg(II), and <sup>198</sup>Hg-enriched EtHg to procedural blanks. For the blank values calculation, we considered a theoretical sample weight of 0.5 g. Detection and quantification limits were calculated as three- and ten-times, respectively, the standard deviation of six procedural blanks. Blanks were analysed by three different mass spectrometers: i) Agilent 7000C triple quadrupole GC-MS/MS operating both in SIM and SRM modes, ii) a GC Agilent 6890 coupled to an Agilent 7500ce ICP-MS, iii) a GC Agilent 7890 coupled to an Agilent 7900 ICP-MS. Table 2 shows the blank values, limits of detection (LOD) and limits of quantification (LOQ) obtained expressed as ng Hg g<sup>-1</sup>. In general, blank values and LODs are lower than 1 ng g<sup>-1</sup> for all mercury compounds and for all mass spectrometric techniques compared. As can be observed, the lowest LODs and LOQs were mostly obtained by the GC-ICP-MS coupling. We did not observe a significant difference in the LOD and LOQ values when using an older Agilent 7500ce or the new Agilent 7900 indicating that, for GC-ICP-MS, the method detection limit is not governed

by instrumental sensitivity. In the case of GC-EI-MS and GC-EI-MS/MS lower LODs and LOQs were obtained in general by SIM compared to the SRM mode. This could be attributed to the low yield of the selected in-cell fragmentation pathway. In summary GC-ICP-MS provided three times lower detection limits for Hg(II) and MeHg than GC-EI-MS and between 4 to 10 times lower than GC-EI-MS/MS. For EtHg detection limits are similar for GC-ICP-MS and GC-EI-MS. In conclusion, procedural blank values and detection limits are low enough by all mass spectrometric techniques evaluated to be applied to the analysis of real samples where other problems, such as spectral interferences or matrix effects, could hinder the application of some techniques in some matrices. So, the analysis of reference materials needs to be performed to evaluate the real applicability range.

**Table S5.** Blank values, limits of detection (LOD) and limits of quantification (LOQ) expressed as ng Hg g<sup>-1</sup> obtained for Hg(II), MeHg and EtHg from the determination of n=6 independent blanks considering a sample amount of 0.5g using four different mass spectrometric techniques.

	Mass Spectrometer	Technique	B1	B2	B3	B4	B5	B6	Average	St. Dev	LOD	LOQ
Hg(II)	Agilent 7000B	GC-EI-MS	0.05	0.08	0.32	0.29	0.17	0.26	0.20	0.11	<b>0.34</b>	<b>1.12</b>
	Agilent 7000B	GC-EI-MS/MS	0.42	0.45	0.07	0.34	0.52	0.20	0.33	0.17	<b>0.51</b>	<b>1.71</b>
	Agilent 7500c	GC-ICP-MS	0.10	0.09	0.19	0.14	0.17	0.13	0.14	0.04	<b>0.12</b>	<b>0.39</b>
	Agilent 7900	GC-ICP-MS	0.04	0.11	0.13	0.08	0.14	0.12	0.10	0.04	<b>0.11</b>	<b>0.37</b>
MeHg	Agilent 7000B	GC-EI-MS	0.02	0.03	0.01	0.02	0.03	0.09	0.04	0.03	<b>0.09</b>	<b>0.28</b>
	Agilent 7000B	GC-EI-MS/MS	0.09	0.21	0.04	0.23	0.24	0.01	0.14	0.10	<b>0.30</b>	<b>1.01</b>
	Agilent 7500c	GC-ICP-MS	0.05	0.05	0.06	0.06	0.05	0.06	0.06	0.01	<b>0.02</b>	<b>0.05</b>
	Agilent 7900	GC-ICP-MS	0.09	0.10	0.10	0.11	0.09	0.10	0.10	0.01	<b>0.02</b>	<b>0.06</b>
EtHg	Agilent 7000B	GC-EI-MS	0.04	0.07	0.02	0.01	0.02	0.04	0.03	0.02	<b>0.07</b>	<b>0.23</b>
	Agilent 7000B	GC-EI-MS/MS	0.00	0.00	-0.06	0.19	0.08	0.07	0.05	0.09	<b>0.26</b>	<b>0.86</b>
	Agilent 7500c	GC-ICP-MS	0.12	0.18	0.20	0.21	0.20	0.22	0.19	0.04	<b>0.11</b>	<b>0.36</b>
	Agilent 7900	GC-ICP-MS	0.05	0.06	0.05	0.05	0.05	0.06	0.05	0.01	<b>0.02</b>	<b>0.06</b>



**Table S6.** Results obtained in the analysis of the Certified reference materials NIST SRM 955c, IAEA-085, IAEA-086 and DOLT-4 by GC-ICP-MS, GC-EI-MS (SIM Mode) and GC-EI-MS/MS (SRM Mode). Concentrations for MeHg, EtHg, Hg(II) and total Hg (THg) are expressed as  $\mu\text{g Hg g}^{-1}$  except for NIST SRM 955c ( $\text{ng Hg g}^{-1}$ ). Uncertainty of the values correspond to the standard deviation of n=3 independent replicates.

		MeHg	EtHg	Hg(II)	THg
IAEA-085	GC-ICP-MS	23.35 ± 0.29	---	0.77 ± 0.01	24.13 ± 0.29
	GC-EI-MS	23.07 ± 0.21	--	0.68 ± 0.02	23.75 ± 0.22
	GC-EI-MS/MS	21.95 ± 0.22	--	0.70 ± 0.03	22.66 ± 0.24
	<i>Certified values</i>	<b>22.90 ± 1.00</b>	-	-	<b>23.20 ± 0.80</b>
IAEA-086	GC-ICP-MS	0.28 ± 0.01	--	0.27 ± 0.01	0.56 ± 0.01
	GC-EI-MS	0.29 ± 0.01	--	0.26 ± 0.01	0.55 ± 0.02
	GC-EI-MS/MS	0.25 ± 0.02	--	0.25 ± 0.00	0.50 ± 0.02
	<i>Certified values</i>	<b>0.26 ± 0.02</b>	-	-	<b>0.57 ± 0.04</b>
DOLT-4	GC-ICP-MS	1.34 ± 0.02	--	1.38 ± 0.08	2.72 ± 0.08
	GC-EI-MS	1.30 ± 0.02	--	1.16 ± 0.03	2.46 ± 0.04
	GC-EI-MS/MS	1.19 ± 0.02	---	1.12 ± 0.06	2.31 ± 0.07
	<i>Certified values</i>	<b>1.33 ± 0.12</b>	-	-	<b>2.58 ± 0.22</b>
NIST SRM 955c Level 4	GC-ICP-MS	6.58 ± 0.16	7.36 ± 0.18	18.72 ± 0.62	32.66 ± 0.66
	GC-EI-MS	8.50 ± 2.68	8.65 ± 1.00	19.46 ± 3.41	36.61 ± 2.43
	GC-EI-MS/MS	5.32 ± 2.34	5.90 ± 1.39	18.73 ± 2.58	29.96 ± 5.10
	<i>Reference values</i>	<b>7.70 ± 0.37</b>	<b>9.4 ± 3.9</b>	<b>18.4 ± 5.2</b>	<b>35.40 ± 2.0</b>

**Table S7.** Range of interconversion factors obtained in interconversion factors (%) obtained in the analysis of the certified reference materials NIST SRM 955c, IAEA-085, IAEA-086 and DOLT-4 by GC-ICP-MS, GC-EI-MS (SIM Mode) and GC-EI-MS/MS (SRM Mode). The factors F3 to F6 involving EtHg are only relevant for NIST SRM 955c.

		F1 MeHg →Hg(II)	F2 Hg(II) -->MeHg	F3 EtHg--> Hg(II)	F4 Hg(II)--> EtHg	F5 MeHg--> EtHg	F6 EtHg--> MeHg
IAEA-085	GC-ICP-MS	1.2 – 2.5	-2.2 – 2.7	-	-	-	-
	GC-EI-MS	1.8 – 2.6	-3.5 – -0.1	-	-	-	-
	GC-EI-MSMS	1.5 – 3.0	-3.3 – 7.9	-	-	-	-
IAEA-086	GC-ICP-MS	2.5 – 3.7	-0.5 – 0.0	-	-	-	-
	GC-EI-MS	2.2 – 3.6	1.7 – 0.7	-	-	-	-
	GC-EI-MSMS	-0.2 – 4.7	-1.7 – 0.9	-	-	-	-
DOLT-4	GC-ICP-MS	1.7 – 4.8	0.0 – 2.8	-	-	-	-
	GC-EI-MS	1.0 – 4.8	-3.4 – -1.3	-	-	-	-
	GC-EI-MSMS	-0.1 – 5.3	-4.0 – 1.8	-	-	-	-
NIST SRM 955c Level 4	GC-ICP-MS	2.5 – 9.5	-0.1 - 0.1	28.5 – 68.0	-0.1 – 0.2	-0.1 - 0.5	-0.1 – 0.4
	GC-EI-MS	2.6 – 11.7	-1.1 – 0.98	35.1 – 65.7	-0.1 – 1.2	0.0 – 0.8	-0.4 – -5.5
	GC-EI-MSMS	-2.3 – 9.7	0.0 – 1.6	45.6 – 71.7	0.0 – 0.3	0.1 – 0.9	-1.1 – 3.0