Electronic Supplementary Information for

The molecular form of mercury in biota: Identification of novel mercury peptide complexes in plants

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Table S1. Instrument parameters

ICP-MS		**Agilent 7500 c**
Torch		Standard
Ar Gas flows:	Cool Gas	16 L/min
	Auxiliary Gas	1 L/min
	Nebulizer Gas	0.95 L/min
	Optional Gas O_2	5 %
Spray chamber		Scott, cooled (2 °C)
Nebulizer		PFA, microconcentric
Internal standard		Continuous aspiration, 20 µg/L Rh in 1% HNO_3
Cones		Platinum
Isotopes monitored		^{200}Hg, ^{202}Hg, ^{103}Rh, ^{34}S, $^{32}S^{16}O$ (m/z 48)
ES-MS (low resolution)		**Agilent XCT ion trap**
Ion source		ESI
Capillary voltage		4500 V
Nebuliser Pressure		50 psi
Drying gas		12 L/min
Gas Temperature		350 °C
Scan window		m/z 200 – m/z 1200
ES-MS (high resolution)		**Thermo Finnigan OrbiTrap**
Ion source		ESI
Capillary Temperature		320 °C
Scan window		m/z 300 – m/z 1200
Resolution		30.000
HPLC parameters*		**Agilent 1100 / Thermo Accela**
Column		Agilent Zorbax Eclipse XDB C-18 (4.6 x 150 mm)
Eluent flow		1 mL/min
Injection volume		100 µL
Eluent A		0.1 % Formic Acid in H_2O
Eluent B		0.1% Formic Acid in Methanol
Gradient program	time	% A / % B
	0	99.5 / 0.5
	10	85 / 15
	12	85 / 15
	12.01	85 / 15
	16	50 / 50
	30	50 / 50
	30.01	99.5 / 0.5
	40	99.5 / 0.5

* The HPLC effluent was split using a micro flow splitter (Upchurch, UK) into 80% (ES-MS) and 20 % (ICP-MS) when the Agilent system was used. A split of 75 % into the ES-MS was used when the Thermo system was employed. When the gradient program for HPLC was elevated to 100 % Methanol/formic acid after 30 minutes, no other mercury compounds were eluted from the system, therefore the initial gradient was used subsequently. Only the Agilent systems were used for simultaneous ICP-MS and ES-MS measurements.

Table S2: Molecular formulae and protonated accurate masses for the mercury phytochelatin complexes found in *Oriza sativa* exposed to Hg^{2+}, and mercury glutathione (GS_2Hg) standard

Name	Formula (H+)	202Hg (theor.)*	202Hg (exp.)	Δ ppm
GS_2Hg	$C_{20}H_{33}N_6O_{12}HgS_2$	815.1304	815.13076	-0.4416
(Ser)PC_2Hg	$C_{19}H_{30}N_5O_{11}HgS_2$	770.1089	770.10748	1.8439
(des-Gly)PC_2Hg	$C_{16}H_{25}N_4O_9HgS_2$	683.0769	683.07631	0.8637
PC_2Hg	$C_{18}H_{28}N_5O_{10}HgS_2$	740.0984	740.0981	0.4054
(Glu)PC_2Hg	$C_{21}H_{32}N_5O_{12}HgS_2$	812.1195	812.11842	1.3299
		200Hg (theor.)*	200Hg (exp.)	Δ ppm
GS_2Hg	$C_{20}H_{33}N_6O_{12}Hg\ S_2$	813.1281	813.12887	-0.9470
(Ser)PC_2Hg	$C_{19}H_{30}N_5O_{11}HgS_2$	768.1066	768.10527	1.7315
(des-Gly)PC_2Hg	$C_{16}H_{25}N_4O_9HgS_2$	681.0746	681.07412	0.7048
PC_2Hg	$C_{18}H_{28}N_5O_{10}HgS_2$	738.0961	738.09578	0.4336
(Glu)PC_2Hg	$C_{21}H_{32}N_5O_{12}HgS_2$	810.1172	810.11578	1.7528

* Theoretical masses (high resolution) were calculated using the Isotope Pattern Calculator v.4 by Yunhua Yan accessed via: http://www.geocities.com/junhuayan/pattern.htm Accurate mass determination was obtained using HPLC coupled to OrbiTrap (Thermo Finnigan, Bremen, Germany), HPLC and ES-MS conditions as described in Table S1.

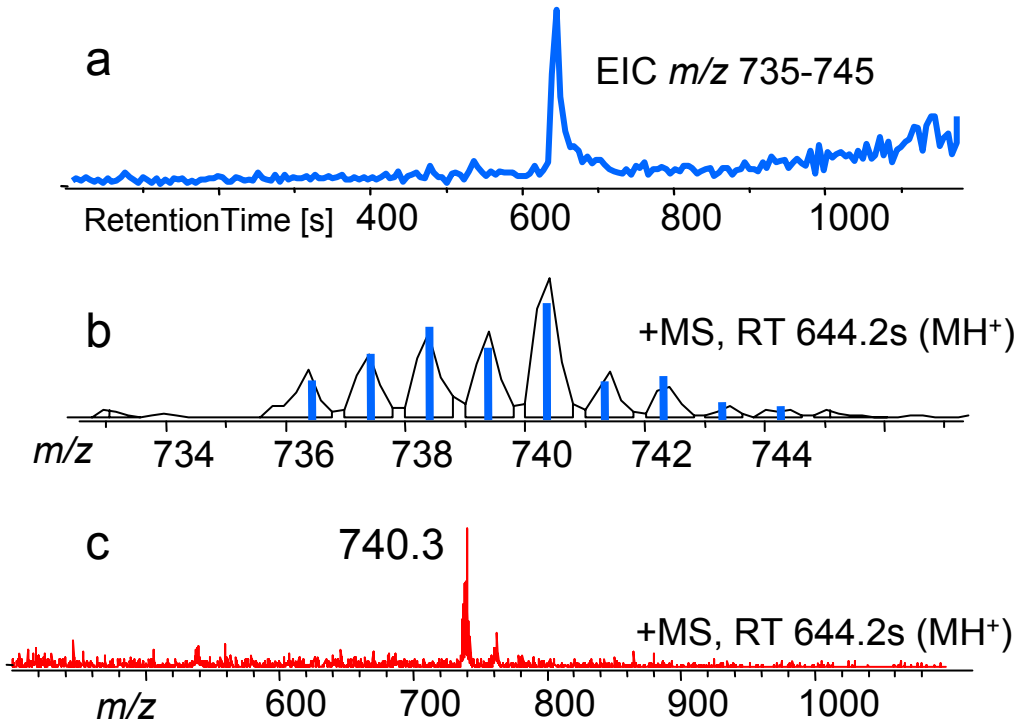

Figure S1: HPLC-ES-MS for a PC_2Hg standard; (Agilent XCT)
a: Extracted ion chromatogram (EIC) for *m/z* 735-745; b: mass spectrum of PC_2Hg, *m/z* 732-747, blue lines indicating the theoretical isotope pattern; c: mass spectrum of PC_2Hg, *m/z* 400-1200, indicating no trace of (des-Gly)PC_2Hg due to in-source fragmentation

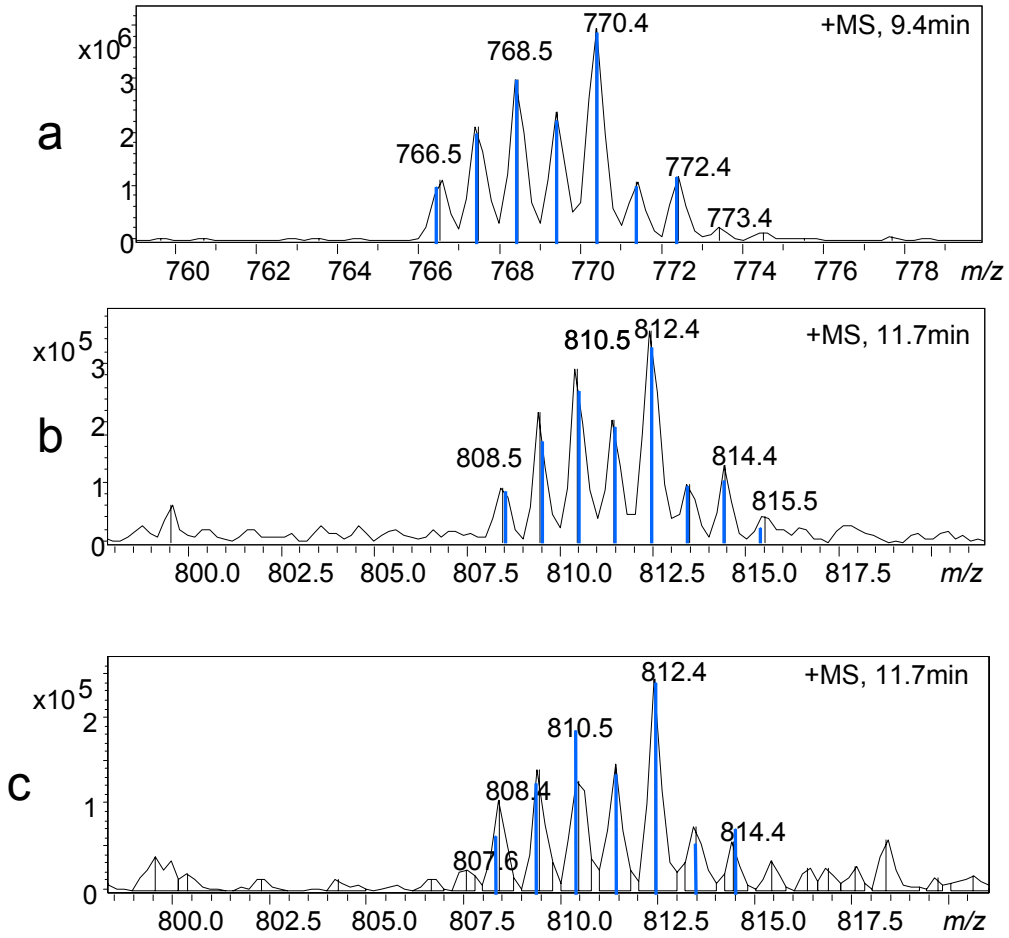

Figure S2: HPLC-ES-MS for extract of *Oriza s.* and *Marrubium v.* roots; blue lines indicating the theoretical isotope pattern
a: (Ser)PC2Hg in roots of *Oriza s.*;
b: (Glu)PC2Hg in roots of *Oriza s.;*
c: (Glu)PC2Hg in roots of *Marrubium v.*;

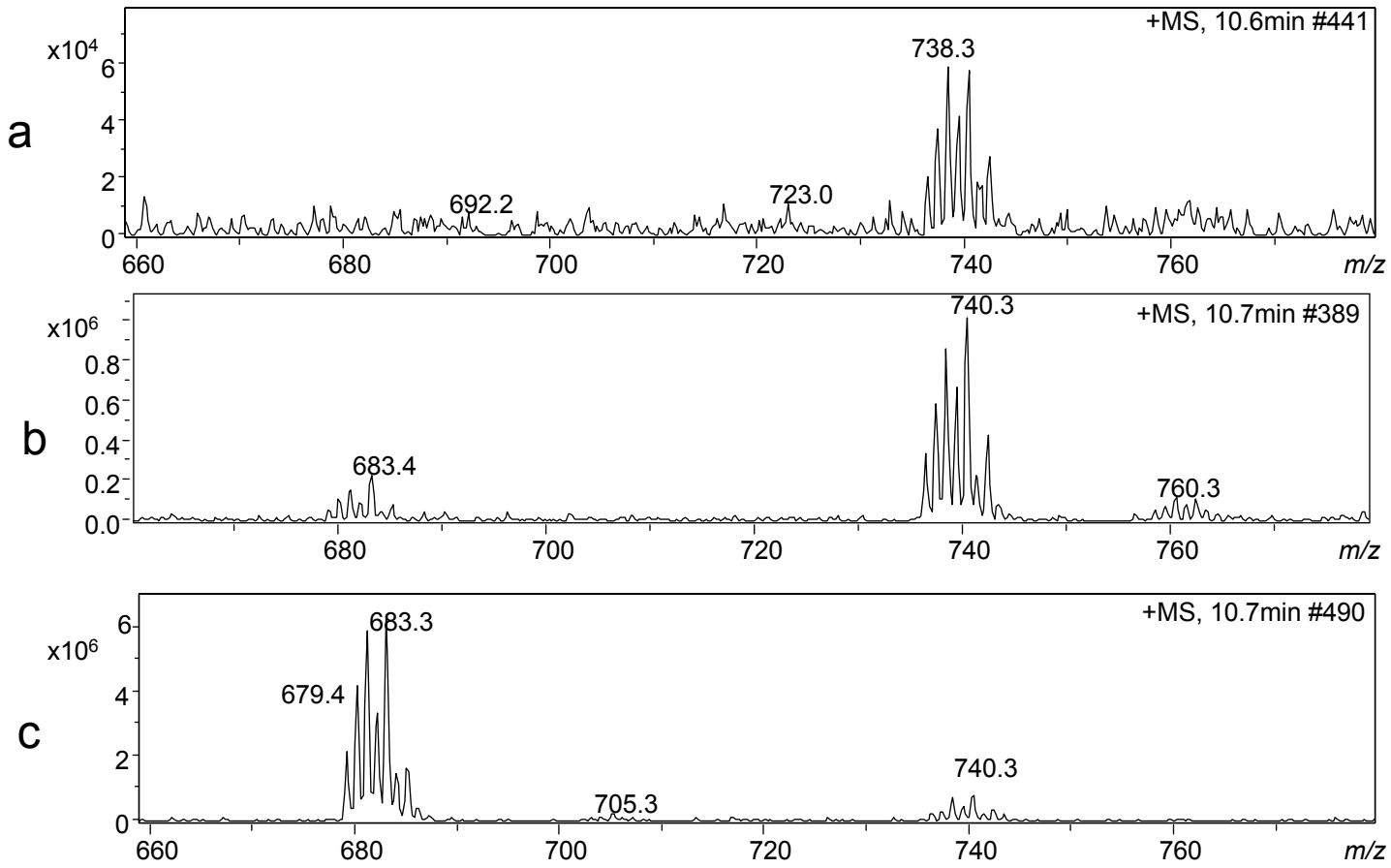

Figure S3: HPLC-ES-MS for extract of *Oriza s.* and *Marrubium v.* roots; @ RT 620s
a: Synthetic PC_2Hg standard;
b: PC_2Hg and (des-Gly)PC_2Hg in roots of *Marrubium v.*
c: PC_2Hg and (des-Gly)PC_2Hg in roots of *Oriza s.*