Electronic supplementary information

Selenium species-dependent toxicity, bioavailability and metabolic transformations in *Caenorhabditis elegans*

Isabelle Rohn, Talke Anu Marschall, Nina Kroepfl, Kenneth Bendix Jensen, Michael Aschner, Simon Tuck, Doris Kuehnelt, Tanja Schwerdtle, and Julia Bornhorst

Table S-1 Selenium species detected in lysates of *C. elegans* following treatment with SeMet or MeSeCys

species	retention time [min]	detected after SeMet treatment[a]	detected after MeSeCys treatment[a]	proposed species	elemental composition	theoretical mass of $[M+H]^+/M^+$ *m/z*	measured mass *m/z*	difference [ppm]	theoretical relative intensity [%]	measured relative intensity [%]
1	5.1	yes, possibly SeOMet	yes	unknown	-	-	-	-	-	-
2	6.4	yes	yes	Se-methylselenocysteine	$C_4H_9NO_2Se$	177.9931	not detected	-	1.8	-
				(MeSeCys)[b]		179.9898	179.9897	-0.8	18.9	15.5
						180.9905	180.9902	-1.9	15.4	13.1
						181.9879	181.9874	-2.9	48.0	45.0
						182.9913	not detected	-	2.1	-
						183.9871	**183.9866**	**-2.8**	**100**	**100**
						184.9905	184.9908	1.8	4.4	2.0
						185.9873	185.9870	-1.3	17.6	17.9
						186.9906	not detected	-	0.8	-
3	8.3	yes	yes	selenomethionine (SeMet)	$C_5H_{11}NO_2Se$	-	-	-	-	-
4	9.8	yes	yes	γ-glutamyl-methylselenocysteine	$C_9H_{16}N_2O_5Se$	307.0357	307.0349	-2.5	1.8	1.4
				(γ-Glu-MeSeCys)[c]		309.0324	309.0322	-0.5	18.9	17.4
						310.0333	310.0330	-0.8	16.1	15.3
						311.0305	311.0309	1.2	48.0	43.8
						312.0339	312.0336	-0.9	4.8	4.3
						313.0297	**313.0293**	**-1.4**	**100**	**100**
						314.0332	314.0325	-2.2	10.0	8.9
						315.0299	315.0298	< 0.3	17.8	16.6
						316.0333	316.0333	< 0.3	1.8	1.5
5	11.0	yes	yes	Se-methylselenogluathione	$C_{11}H_{19}N_3O_6Se$	364.0571	364.0570	-0.3	1.8	1.1
				(MeSeGSH)[c]		366.0540	366.0533	-1.8	18.8	16.9
						367.0550	367.0552	0.5	17.5	15.5
						368.0520	368.0527	2.1	49.0	43.3
						369.0554	369.0561	1.9	6.4	5.1
						370.0513	**370.0517**	**1.0**	**100**	**100**
						371.0543	371.0547	1.2	12.9	11.3
						372.0516	372.0516	< 0.3	18.9	16.9
						373.0547	373.0543	-1.1	2.4	1.8

species	retention time [min]	detected after SeMet treatment[a]	detected after MeSeCys treatment[a]	proposed species	elemental composition	theoretical mass of $[M+H]^+/M^+$ *m/z*	measured mass *m/z*	difference [ppm]	theoretical relative intensity [%]	measured relative intensity [%]
6	11.7	yes	yes	unknown	-	-	-	-	-	-
7	12.4	no	yes	unknown	-	-	-	-	-	-
8	13.8	yes	no	Se-adenosylselenomethionine	$C_{15}H_{23}N_6O_5Se^+$	441.0949	441.0951	0.5	1.8	1.6
				(AdoSeMet)[b]		443.0916	443.0922	1.3	18.8	17.6
						444.0927	444.0936	2.0	17.5	16.6
						445.0898	445.0894	-0.9	48.4	46.3
						446.0931	446.0931	< 0.3	8.1	6.9
						447.0890	**447.0890**	**< 0.3**	**100**	**100**
						448.0924	448.0928	0.9	16.7	15.3
						449.0892	449.0886	-1.3	18.3	18.9
						450.0926	450.0923	-0.7	3.0	2.8
9	18.9	yes	yes	unknown	-	-	-	-	-	-
10	20.9	yes	no	unknown	-	-	-	-	-	-
11	22.3	no	yes	S-selenomethyl-glutathione[c]	$C_{11}H_{19}N_3O_6SSe$	398.0259	398.0252	-2.0	18.9	4.4[d]
						399.0266	399.0269	0.7	15.4	3.2[d]
						400.0240	400.0249	2.1	47.9	39.3
						401.0274	not detected	-	5.7	-
						402.0233	**402.0225**	**-1.8**	**100**	**100**
						403.0266	403.0260	-1.5	11.9	1.7[d]
						404.0234	404.0236	0.5	17.6	0.7[d]
						405.0268	not detected	-	2.1	-
12	25.9	yes	no	unknown	-	-	-	-	-	-
13	38.0	yes	no	Se-adenosylselenohomocysteine	$C_{14}H_{20}N_6O_5Se$	429.0759	429.0767	1.9	18.8	1.7[d]
				(AdoSeHcy)[b]		430.0770	430.0775	1.1	17.3	5.3[d]
						431.0741	431.0733	-1.9	48.4	44.9
						432.0775	432.0768	-1.8	7.6	0.4[d]
						433.0734	**433.0731**	**-0.7**	**100**	**100**
						434.0766	434.0750	-3.8	15.6	8.2[d]
						435.0737	435.0739	0.6	18.3	8.9[d]

a … for HPLC/ICPMS chromatograms see Supplementary Fig. S-1

b … MS data obtained from *C. elegans* lysate after SeMet treatment

c … MS data obtained from *C. elegans* lysate after MeSeCys treatment

d … due to the low absolute intensity of this signal its relative intensity does not ideally match the theoretical one

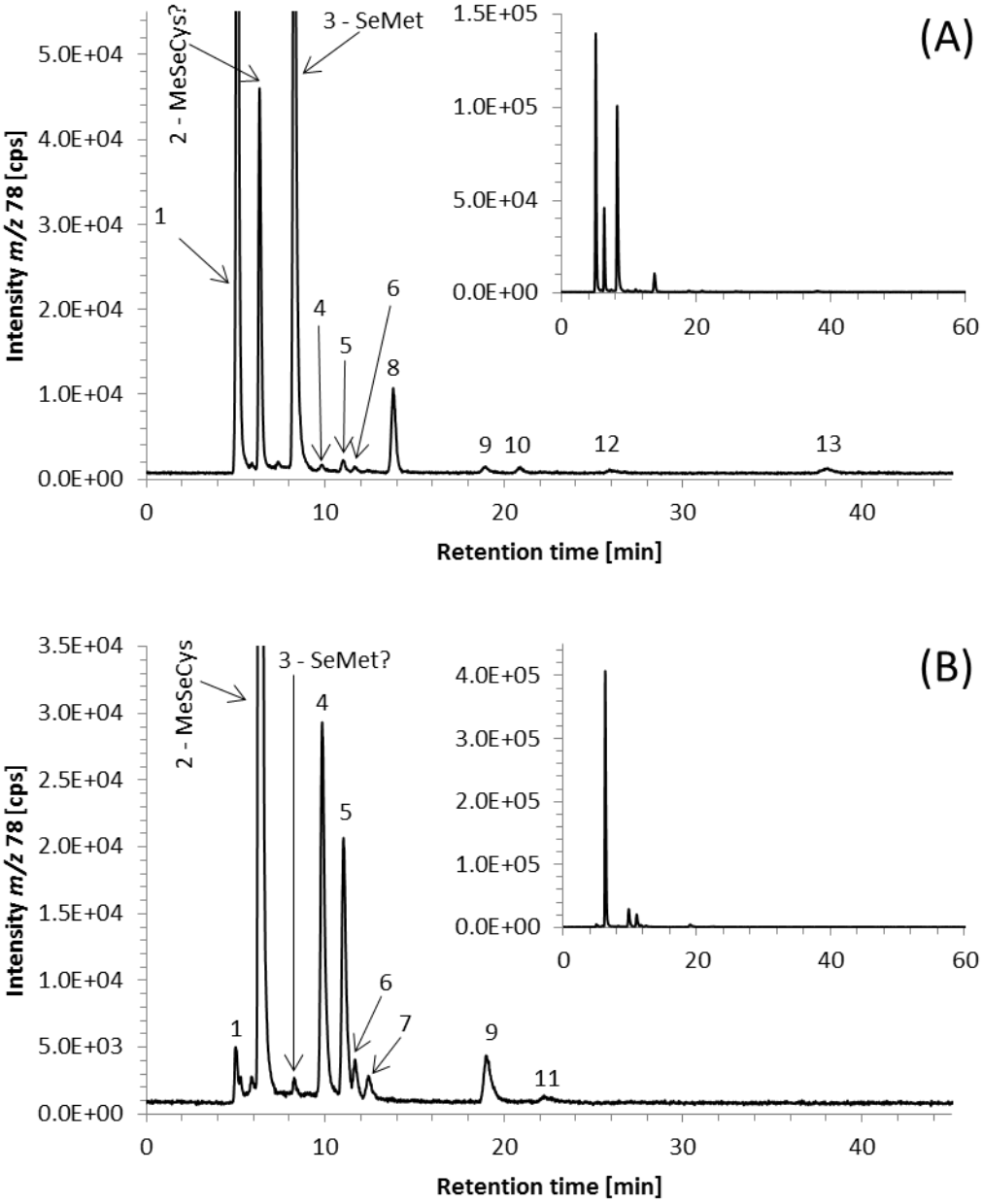

Figure S-1 HPLC/ICPMS chromatograms of *C. elegans* lysates after treatment with (A) SeMet, (B) MeSeCys. These lysates were subsequently investigated by HPLC-ESI-Orbitrap-MS. Chromatographic conditions: YMC-TriartPFP column (3 µm, 3 × 250 mm) at 30°C with 20 mM ammonium formate, 3% MeOH, pH 3 as a mobile phase at a flow rate of 250 µL min^{-1}, injection volume: 5 µL. Detected selenium species were numbered from 1 – 13 according to their retention times. An overview about all detected selenium species is given in Supplementary Table S-1.

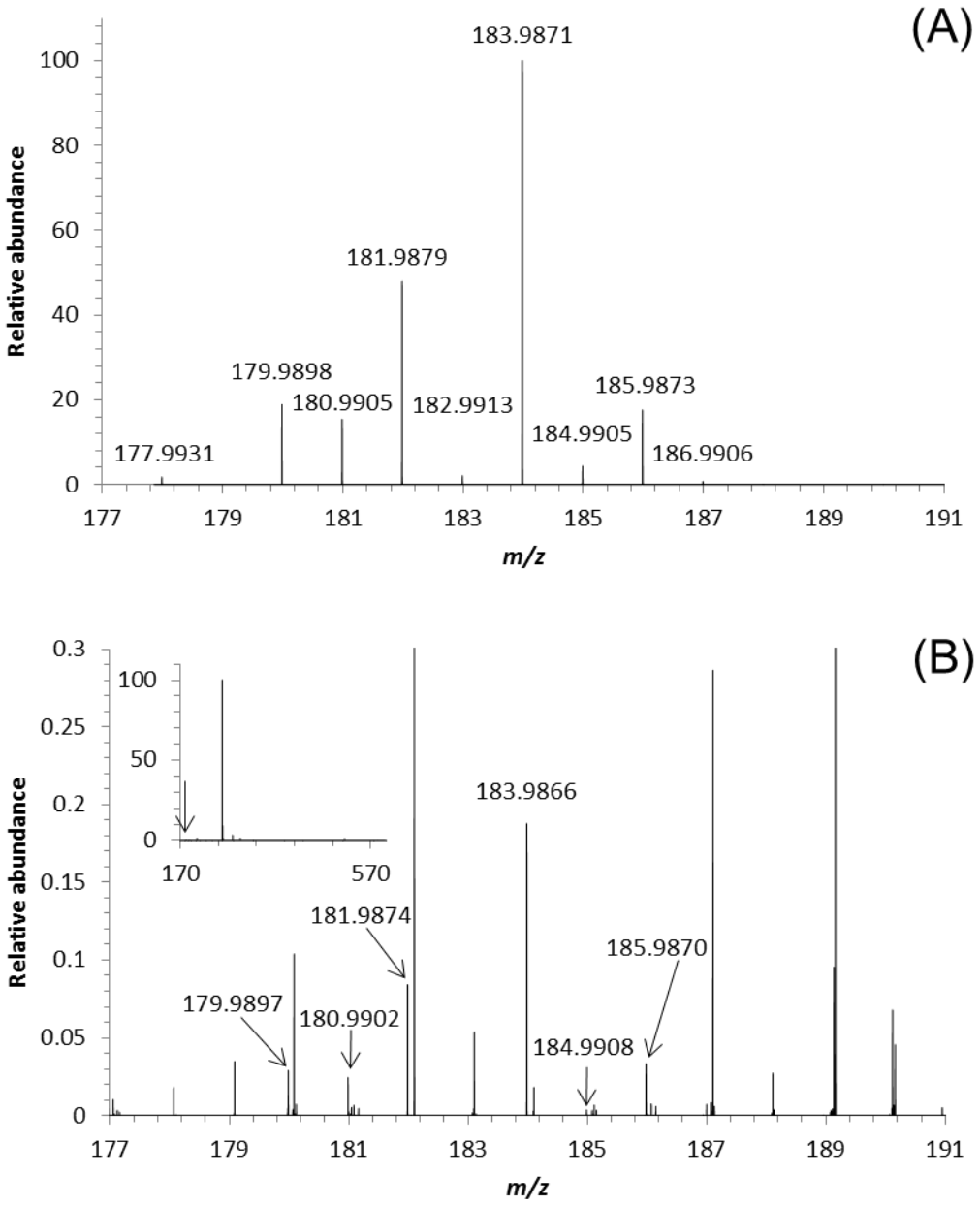

Figure S-2 HPLC-ESI-Orbitrap-MS (background subtracted) of a *C. elegans* lysate following treatment with SeMet: Identification of MeSeCys (species **2**, Supplementary Figure S-1A, Table S-1) *via* its isotope pattern and accurate mass; (A) calculated isotope pattern of MeSeCys, (B) spectrum of MeSeCys in the crude lysate (absolute intensity of the most intense peak in the isotope pattern of the species: 1.3×10^5). For chromatographic conditions see Supplementary Fig. S-1; injection volume: 20 µL.

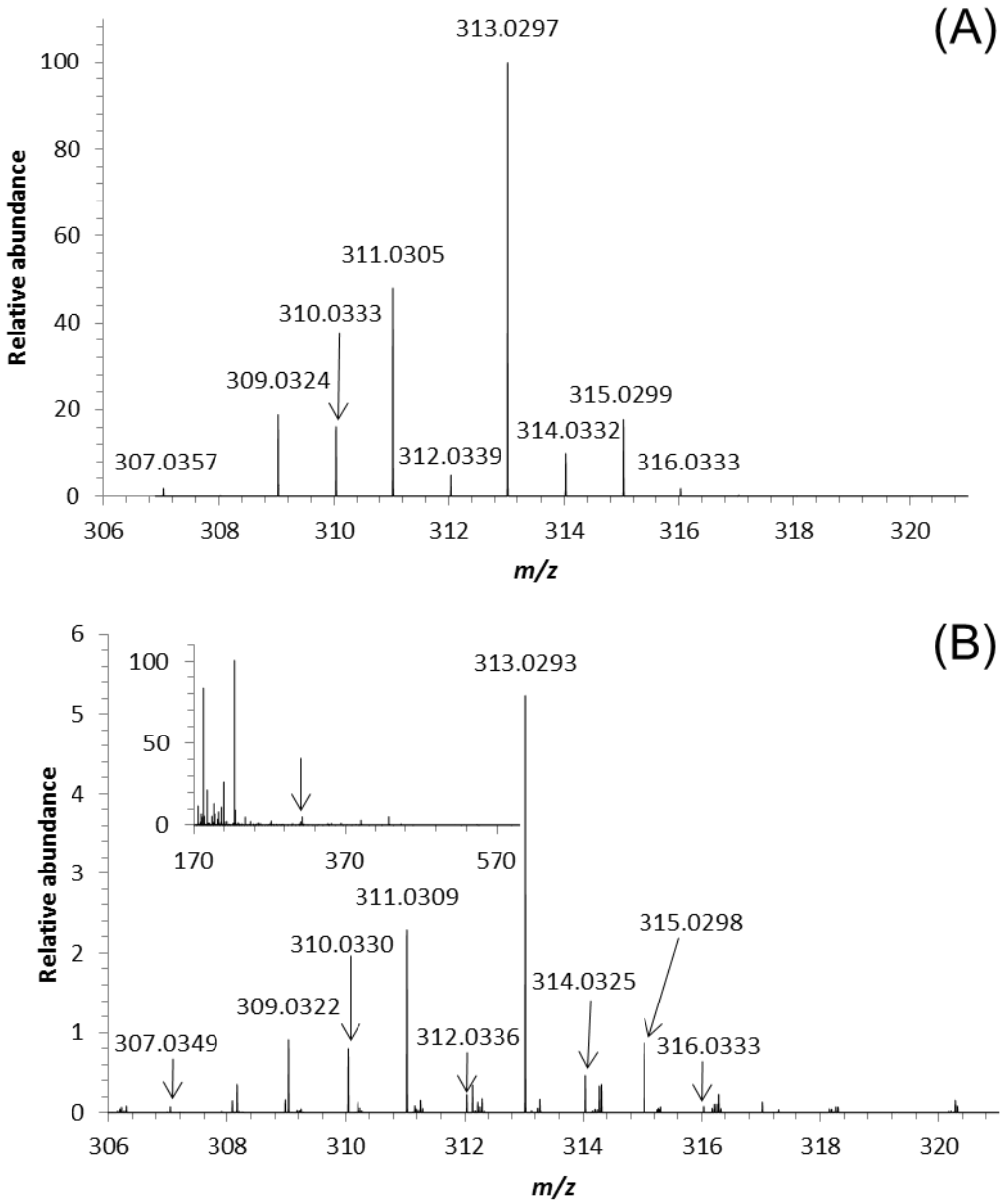

Figure S-3 HPLC-ESI-Orbitrap-MS of a *C. elegans* lysate following treatment with MeSeCys: Identification of γ-Glu-MeSeCys (species **4**, Supplementary Figure S-1B, Table S-1) *via* its isotope pattern and accurate mass; (A) calculated isotope pattern of γ-Glu-MeSeCys, (B) spectrum of γ-Glu-MeSeCys in the crude lysate (absolute intensity of the most intense peak in the isotope pattern of the species: 3.8×10^5). For chromatographic conditions see Supplementary Fig. S-1; injection volume: 20 µL.

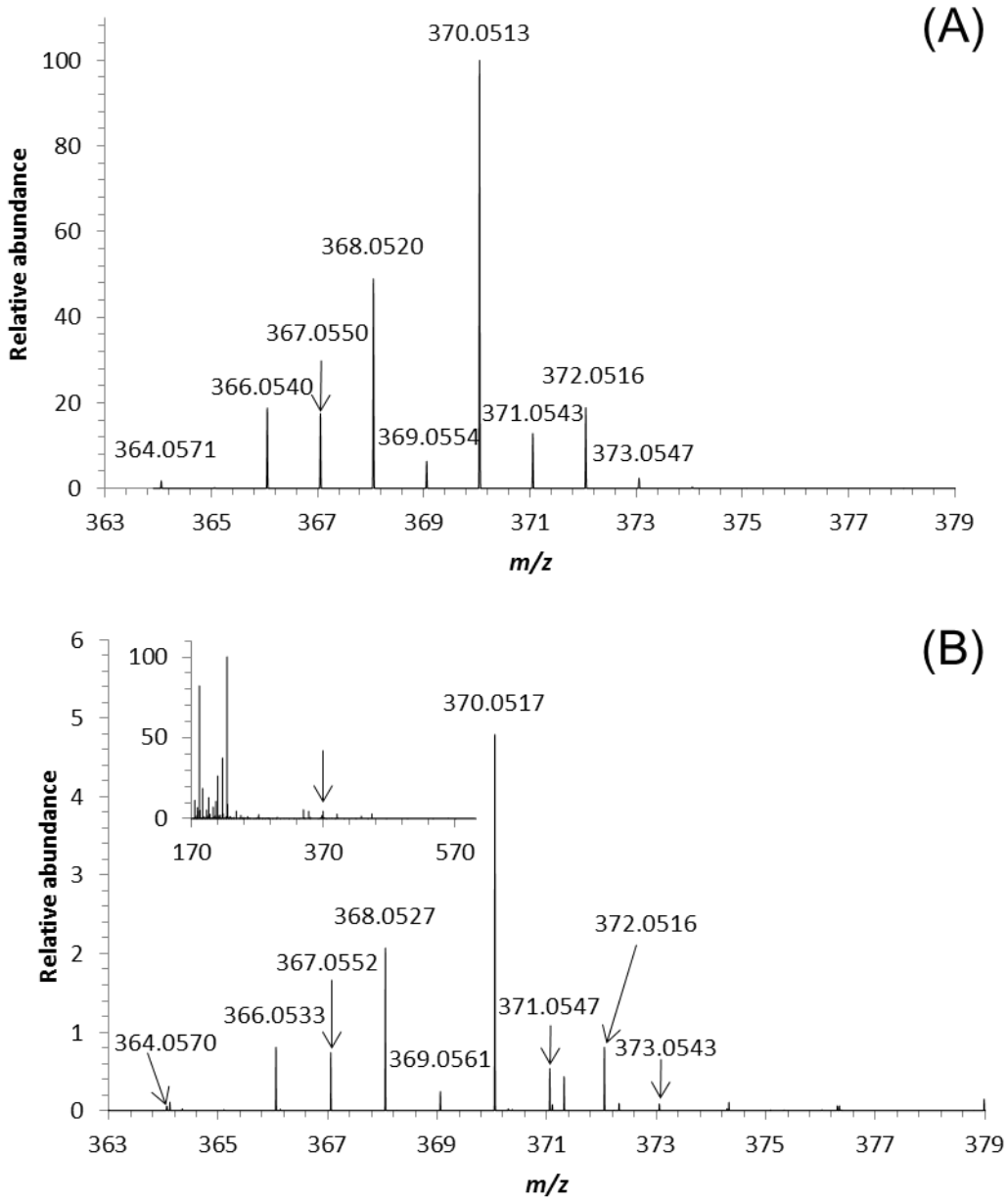

Figure S-4 HPLC-ESI-Orbitrap-MS of a *C. elegans* lysate following treatment with MeSeCys: Identification of MeSeGSH (species **5**, Supplementary Figure S-1B, Table S-1) *via* its isotope pattern and accurate mass; (A) calculated isotope pattern of MeSeGSH, (B) spectrum of MeSeGSH in the crude lysate (absolute intensity of the most intense peak in the isotope pattern of the species: 3.6×10^5). For chromatographic conditions see Supplementary Fig. S-1; injection volume: 20 µL.

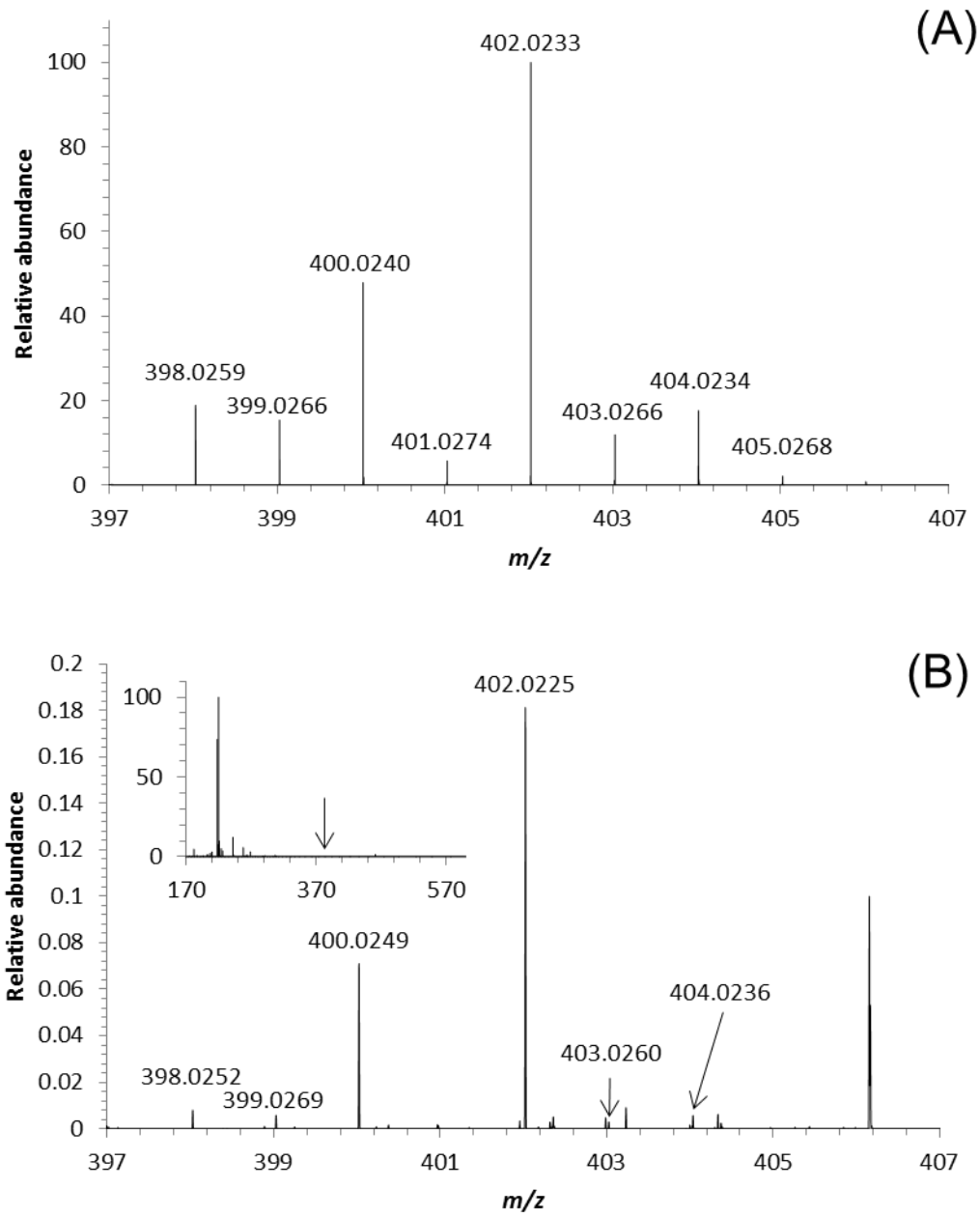

Figure S-5 HPLC-ESI-Orbitrap-MS (background subtracted) of a *C. elegans* lysate following treatment with MeSeCys: Tentative identification of S-selenomethyl-glutathione (species **11**, Supplementary Figure S-1B, Table S-1) *via* its isotope pattern and accurate mass; (A) calculated isotope pattern of S-selenomethyl-glutathione, (B) spectrum of S-selenomethyl-glutathione in the crude lysate (absolute intensity of the most intense peak in the isotope pattern of the species: 7.1×10^3). Due to their low absolute intensities (ca. 1×10^2 to 3×10^2) the less intense isotope signals in the isotope pattern do not ideally match the theoretical abundances. For chromatographic conditions see Supplementary Fig. S-1; injection volume: 20 µL.