elenopeptides and Elemental Selenium in *Thunbergia alata* after Exposed to Selenite: Quantification Method for Elemental Selenium

Fatai Adigun Aborode[1], Andrea Raab[1], Simon Foster[2], Enzo Lombi[3], William Maher[2], Eva M. Krupp[1] and Joerg Feldmann[1]*

Electronic Supplementary Material

Table S1: Description of the proposed fragmentation pattern of the selenocysteinyl-2,3-dihydroxypropionyl glutathione conjugate and the fragment ions following the assigned fragment numbers in Figure 1b.

No	Elemental Composition	Theoretical mass	Measured mass	Error [ppm]	Losses/fragments
1	$C_{16}H_{27}N_4O_{11}SSe^+$	563.0557	563.0548	1.6	Parent compound
2	$C_{14}H_{22}N_3O_9SSe^+$	488.0237	488.0224	2.7	Glycine
3	$C_6H_{10}NO_5Se^+$	255.9719	255.9716	1.2	Glutathione
4	$C_{11}H_{20}N_3O_8SSe^+$	434.0131	434.0119	2.8	γ-Glutamic acid

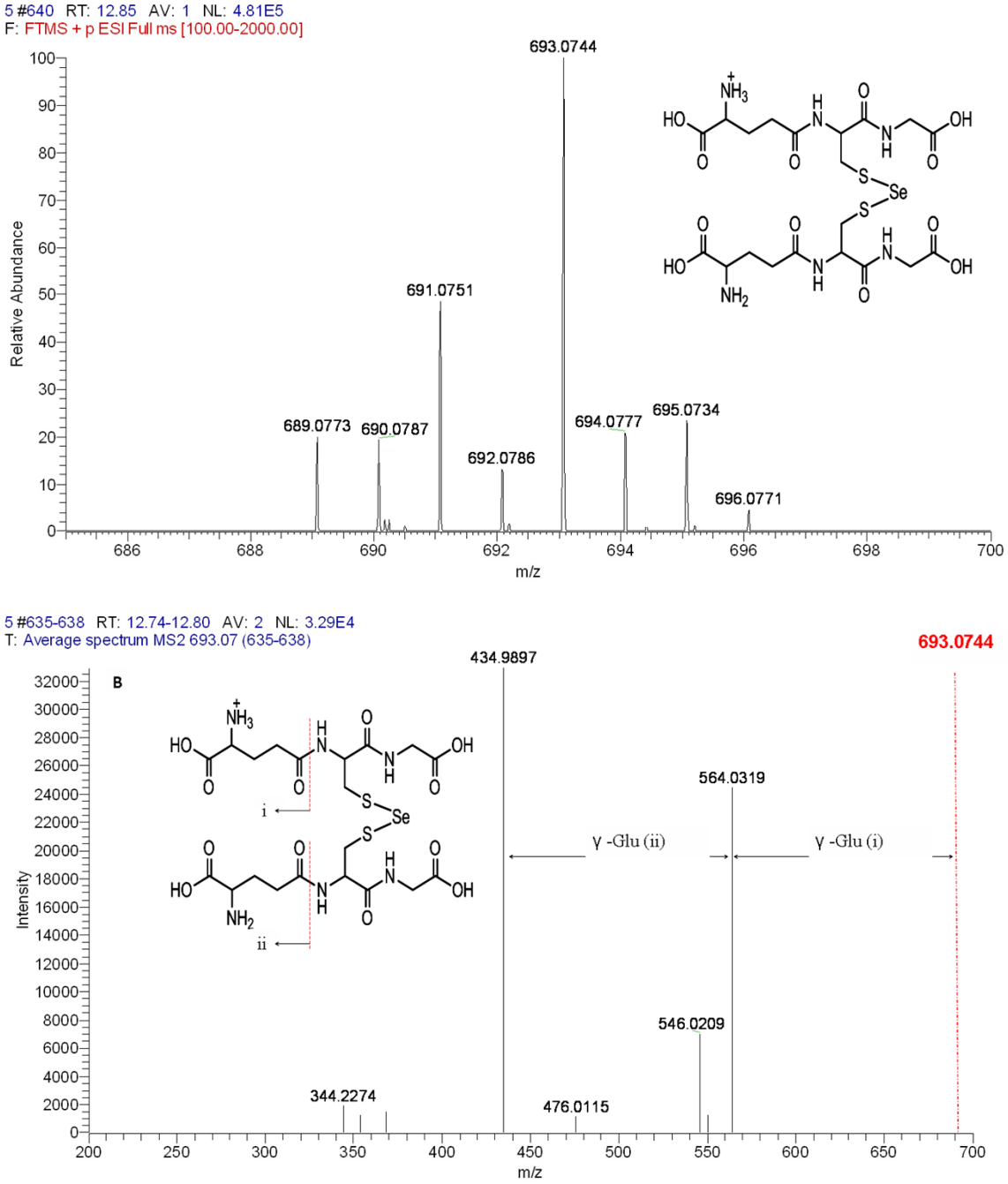

Figure S1: A) ESI-Orbitrap MS of protonated seleno-diglutathione (ESI-MS m/z 693) showing the fingerprint of a mono-selenium isotopic compound for selenite exposed roots, B) MS2 of m/z 693→ (i) m/z 564 corresponding to loss of **γ** - glutamic acid, and (ii) m/z 435 corresponding to loss of additional **γ** - glutamic acid.

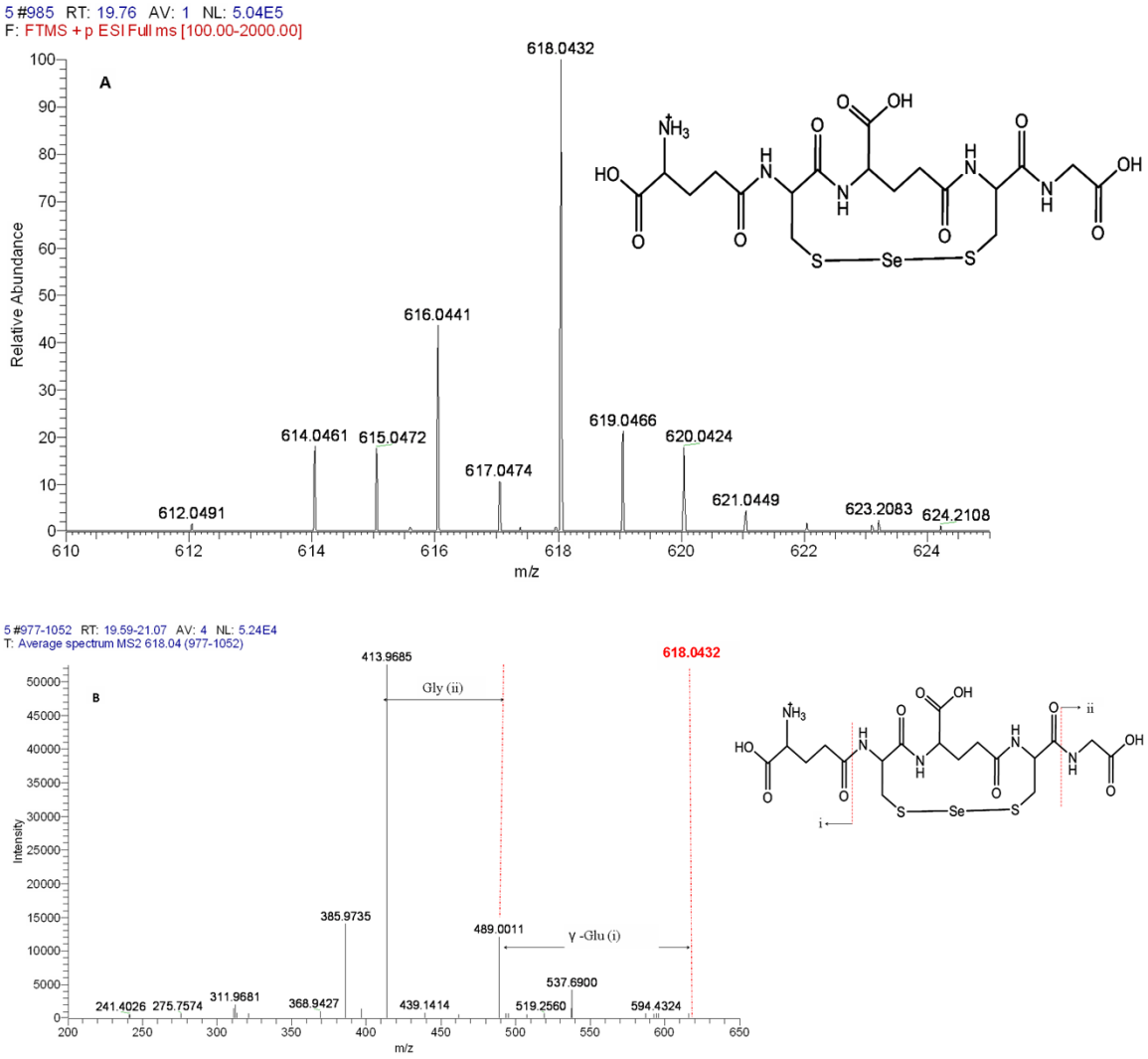

Figure S2: A, ESI-Orbitrap MS of protonated SeII-PC2 (ESI-MS m/z 618) showing the fingerprint of a mono-selenium isotopic compound for selenite exposed roots, B, MS2 of m/z 618→ (i) m/z 489 corresponding to loss of **γ** - glutamic acid, and (ii) m/z 414 corresponding to loss of glycine.

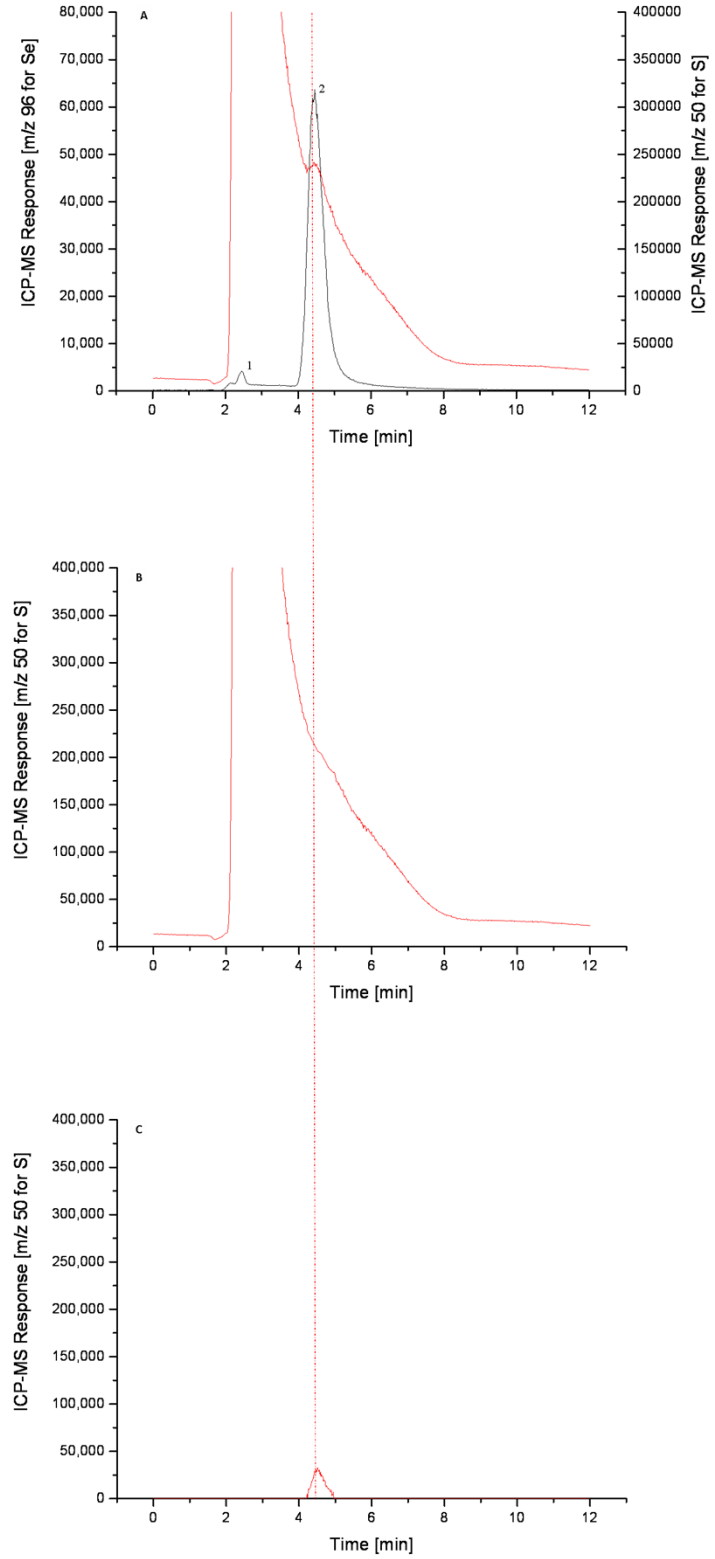

Figure S3: HPLC/ICP-MS Chromatogram of solution representing reactions between formed Nano-Se0 from selenite reduction by GSH and 100 mM sodium sulfite solution. Peak 1 suspected to be contamination in the selenite standard used. A) Co-elution of selenium (peak 2) and sulfur @ 4.5 minutes indicating Se0 eluted as selenosulfate. B) Extrapolated sulfur profile without the tiny peak, C) Sulfur peak residue after removing the extrapolated profile from real sulfur profile in A indicating the additional sulphur signal coeluting with the selenium signal.

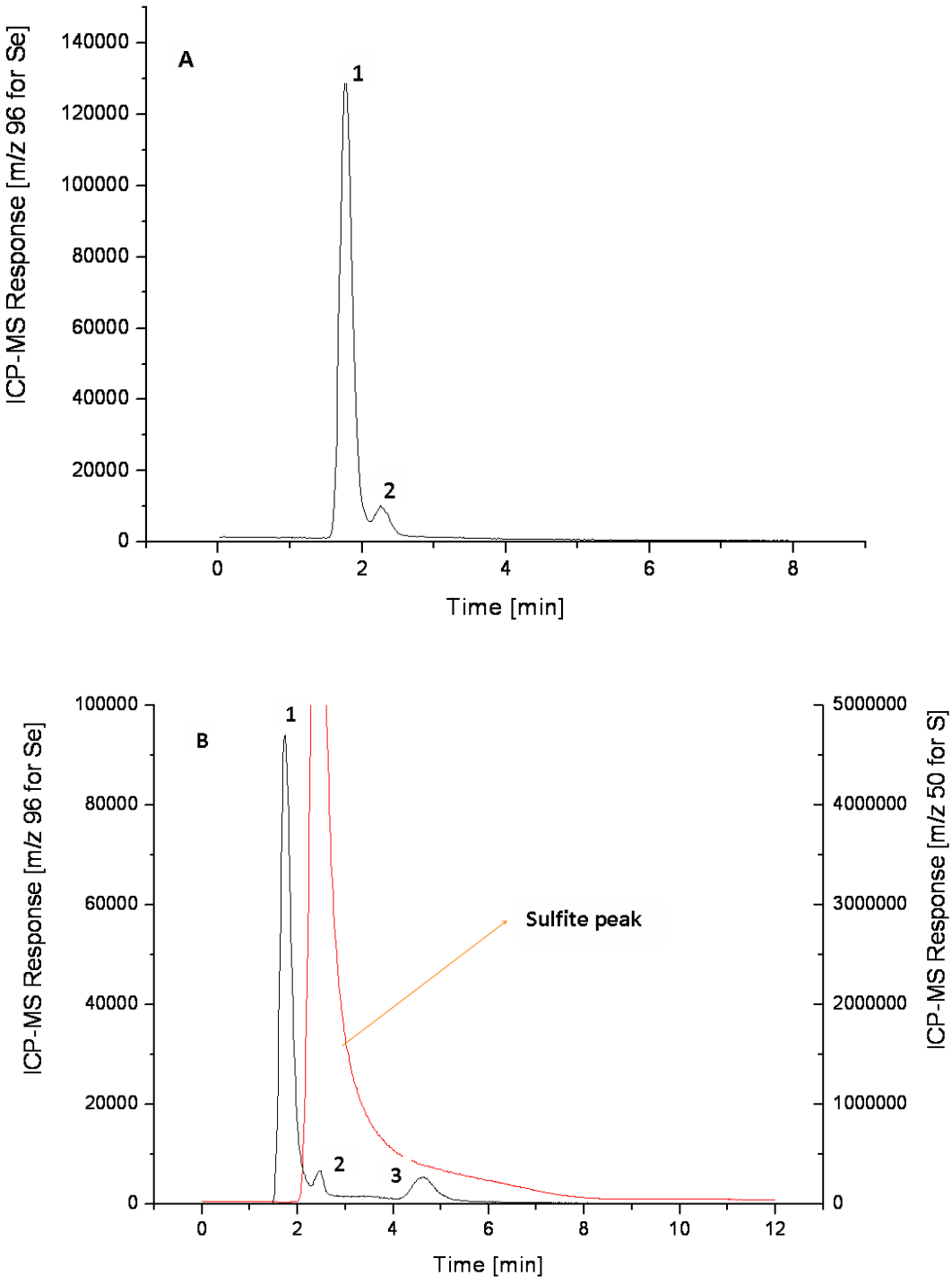

Figure S4 A-B: HPLC/ICP-MS Chromatograms of (A) sodium selenide solution and (B) mixture of sodium selenide with 100 mM sodium sulfite solution. The peaks 1 and 2 in both chromatograms at 1.7 and 2.2 minutes are characteristic of selenite and selenate peaks using this method and the presence of a small peak 3 at 4.5 minutes in chromatogram B indicate that selenosulfate was formed from Se0 produced from oxidation of selenide. The other broad peak in B is from the sulfur signal.

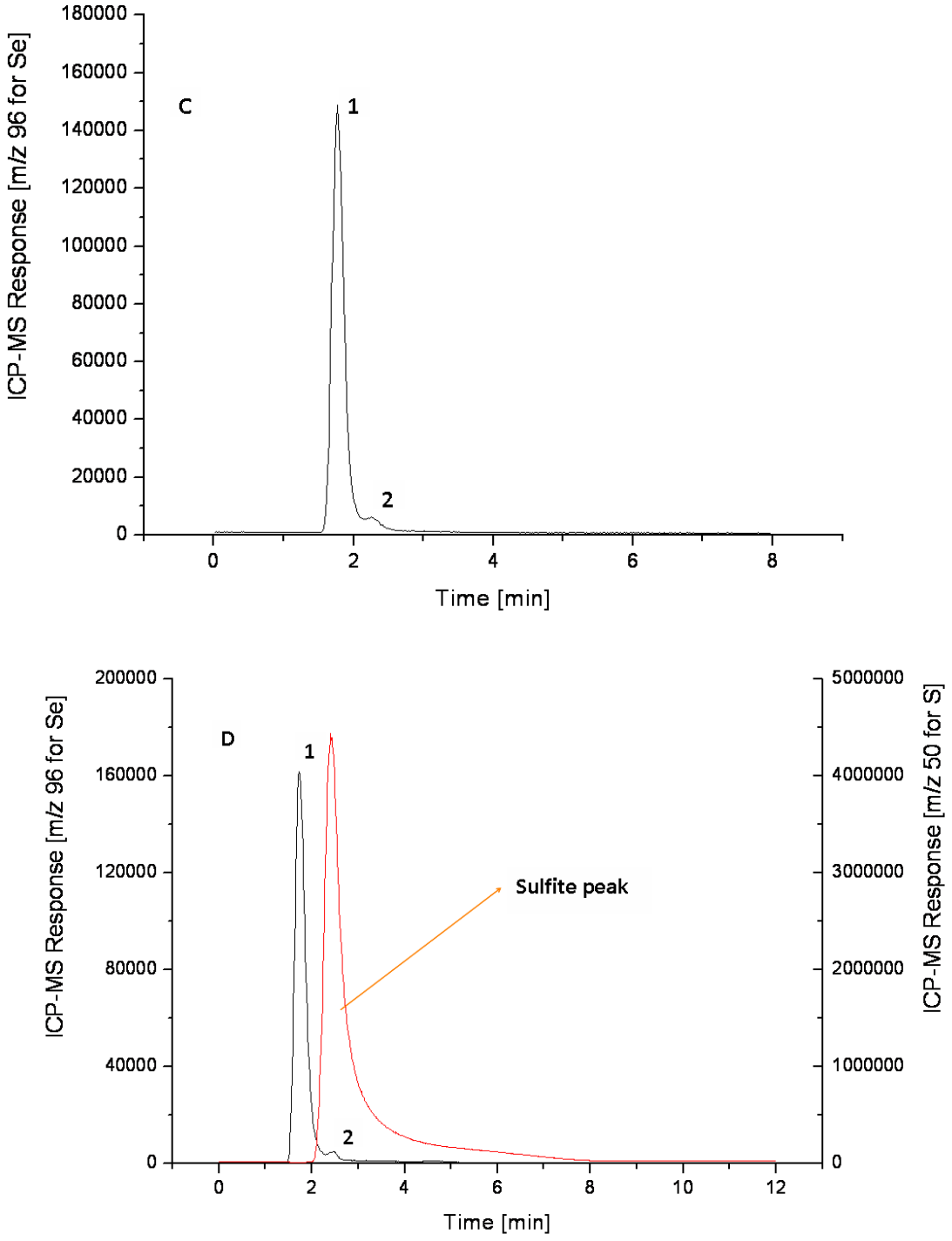

Figure S4 C-D: HPLC/ICP-MS Chromatograms of (C) sodium selenite solution and (D) mixture of sodium selenite with 100 mM sodium sulfite solution. Peak 1 in each chromatogram at 1.7 minutes is characteristic of the selenite peak using this method and the absence of the strong peak at 4.5 minutes in chromatogram D indicates that selenite cannot form selenosulfate with sulfite using this method. Peak 2 is suspected to be from contamination in the selenite standard used. The other broad peak in D is the peak from the sulfur signal and a small peak 2 at 2.3 minutes characteristic of selenate elution.

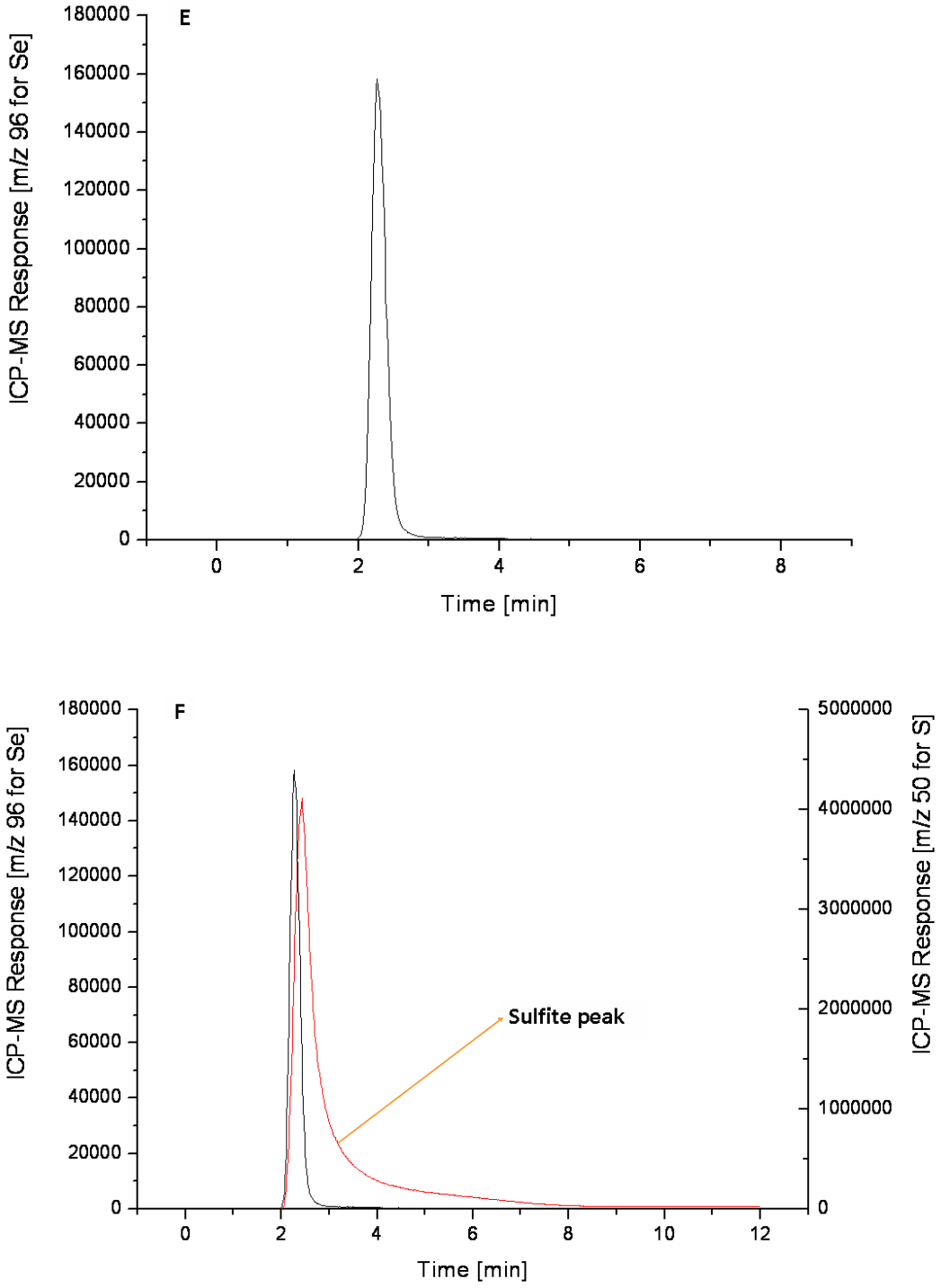

Figure S4 E-F: HPLC/ICP-MS Chromatograms of (E) sodium selenate solution and (F) mixture of sodium selenate with 100 mM sodium sulfite solution. The peak in each chromatogram at 2.3 minutes is characteristic of the selenate peak using this method and the absence of the strong peak at 4.5 minute in chromatogram F indicates that selenate cannot form selenosulfate with sulfite using this method. The other broad peak in F is from the sulfur signal.

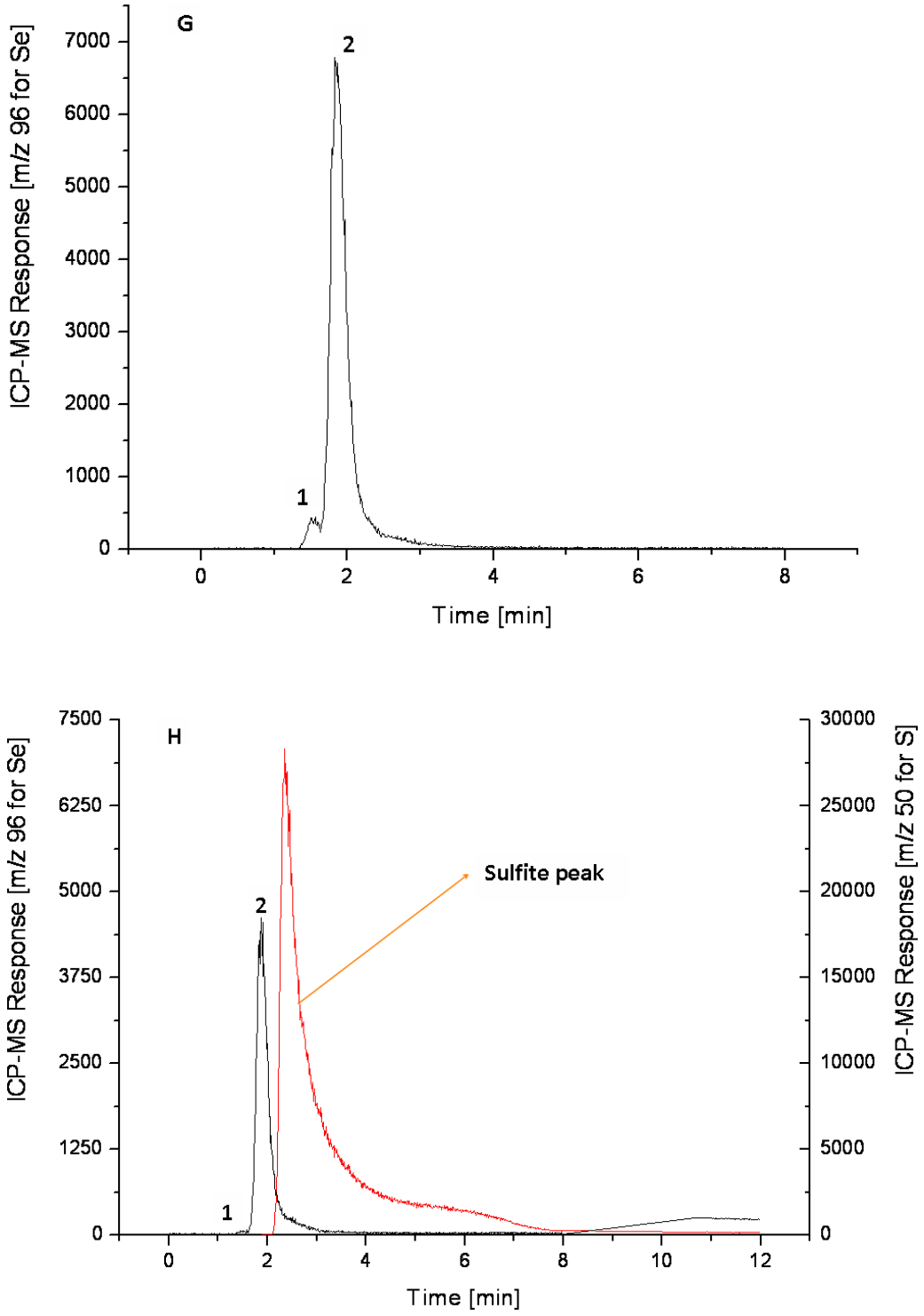

Figure S4 G-H : HPLC/ICP-MS Chromatograms of (G) selenocystine solution and (H) mixture of selenocystine with 100 mM sodium sulfite solution. The peak 2 in both chromatograms at 1.9 minute is for selenocystine elution using this method and the presence of a small peak 1 in both chromatograms indicate contamination in the selenocystine standard. The elution profile on both chromatograms indicated that sulfite did not alter the structure and property of selenocystene. The other broad peak in H is from the sulfur signal.

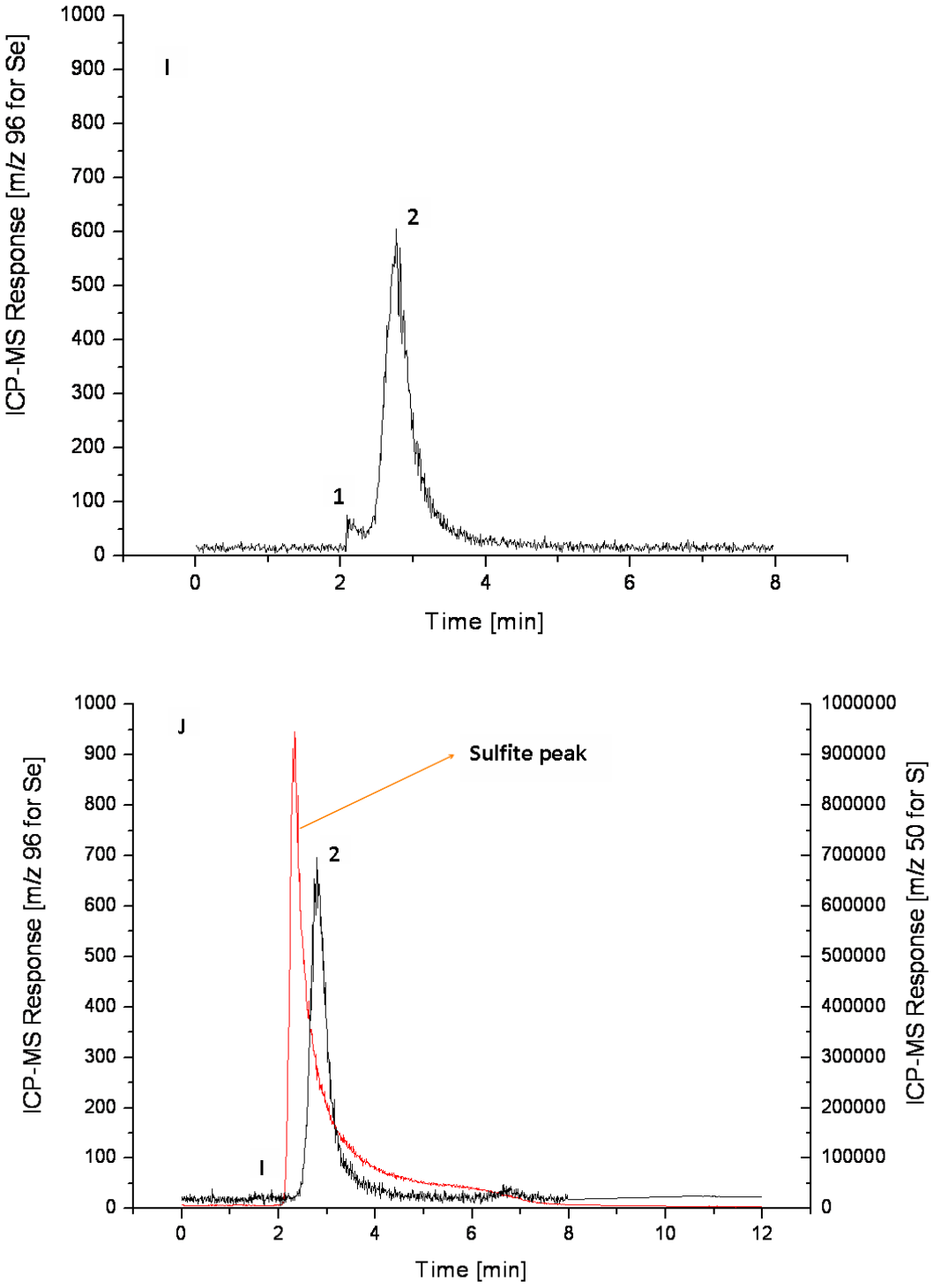

Figure S4 I-J: : HPLC/ICP-MS Chromatograms of (I) selenomethionine solution and (J) mixture of selenomethionine with 100 mM sodium sulfite solution. The strong peak in both chromatograms at 2.8 minutes is for selenomethionine elution using this method and the presence of a small peak next to the main peak in chromatograms indicate contamination in the solution. The elution profile on both chromatograms indicated that sulfite did not alter the structure and property of selenomethionine. The other broad peak in J is from the sulfur signal.