Supplementary material

Selenoneine and ergothioneine in human blood cells determined simultaneously by HPLC/ICP-QQQ-MS

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Typical HPLC and ICP-QQQ-MS settings for the quantitative determination of sulfur and selenium in lysates of human blood cells, high resolution mass spectra of an aqueous lysate of blood cells and external calibration plots.

Table S 1: Typical ICP-QQQ-MS and HPLC settings for the quantitative determination of sulfur and selenium in lysates of human blood cells

Typical ICP-QQQ-MS settings			
RF power	1550 W	*Q1 bias*	-3.0 V
RF matching	1.86 V	*Q1 prefilter bias*	-16.0 V
Sampling depth	8.0 mm	*Q1 postfilter bias*	-14.0 V
Carrier gas flow	0.97 L min^{-1}	*Octopole bias*	-5.0 V
Makeup gas flow	0.00 L min^{-1}	*Octopole RF*	200 V
Optional gas (CO_2 in Ar)	12%	*Cell gas (O_2 in Ar)*	30%
Spray chamber temperature	2°C	*Energy discriminator*	-7.0 V
Extract 1	0.0 V	*Cell focus*	0.0 V
Extract 2	-140.0 V	*Cell entrance*	-50 V
Omega bias	-90 V	*Cell exit*	-60 V
Omega lens	7.0 V	*Deflect*	3.0 V
Q1 entrance	-6.0 V	*Plate bias*	-60 V
Q1 exit	-1.0 V		

HPLC settings		
Column	Atlantis dC18 4.6×150 mm (Waters Corporation, Milford, USA)	
Column temperature	30°C	
Injection volume	10 µL	
Flow rate	1.0 mL min^{-1}	
Mobile phase	20 mM ammonium formate, 3% MeOH, pH 3.0[a]	*(Condition 1 – without TCEP)*
	20 mM ammonium formate, 3% MeOH, 0.1 mM TCEP, pH 3.0[a]	*(Condition 2 – with TCEP)*

[a] pH adjusted with formic acid

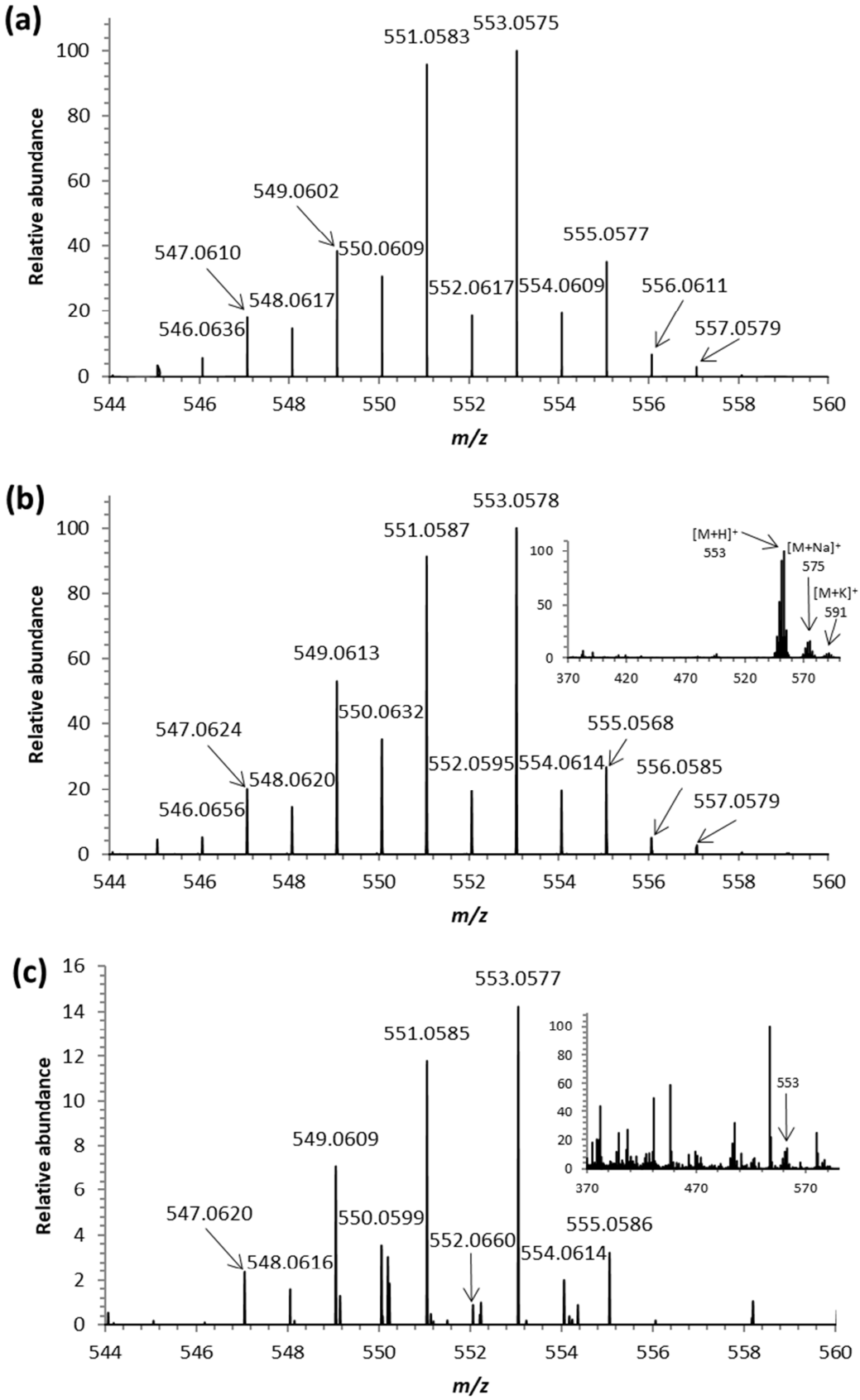

Figure S 1: HPLC/ESI-Orbitrap MS signals of a native lysate of blood cells. (a) simulated spectrum for oxidized selenoneine, (b) full scan spectrum of a 1 mg Se L^{-1} solution of selenoneine isolated from *S. pombe* and (c) background subtracted full scan spectrum of a native lysate of blood cells (volunteer 3). column: Atlantis dC18 4.6 × 150 mm; mobile phase: 20 mM ammonium formate 3% methanol pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL

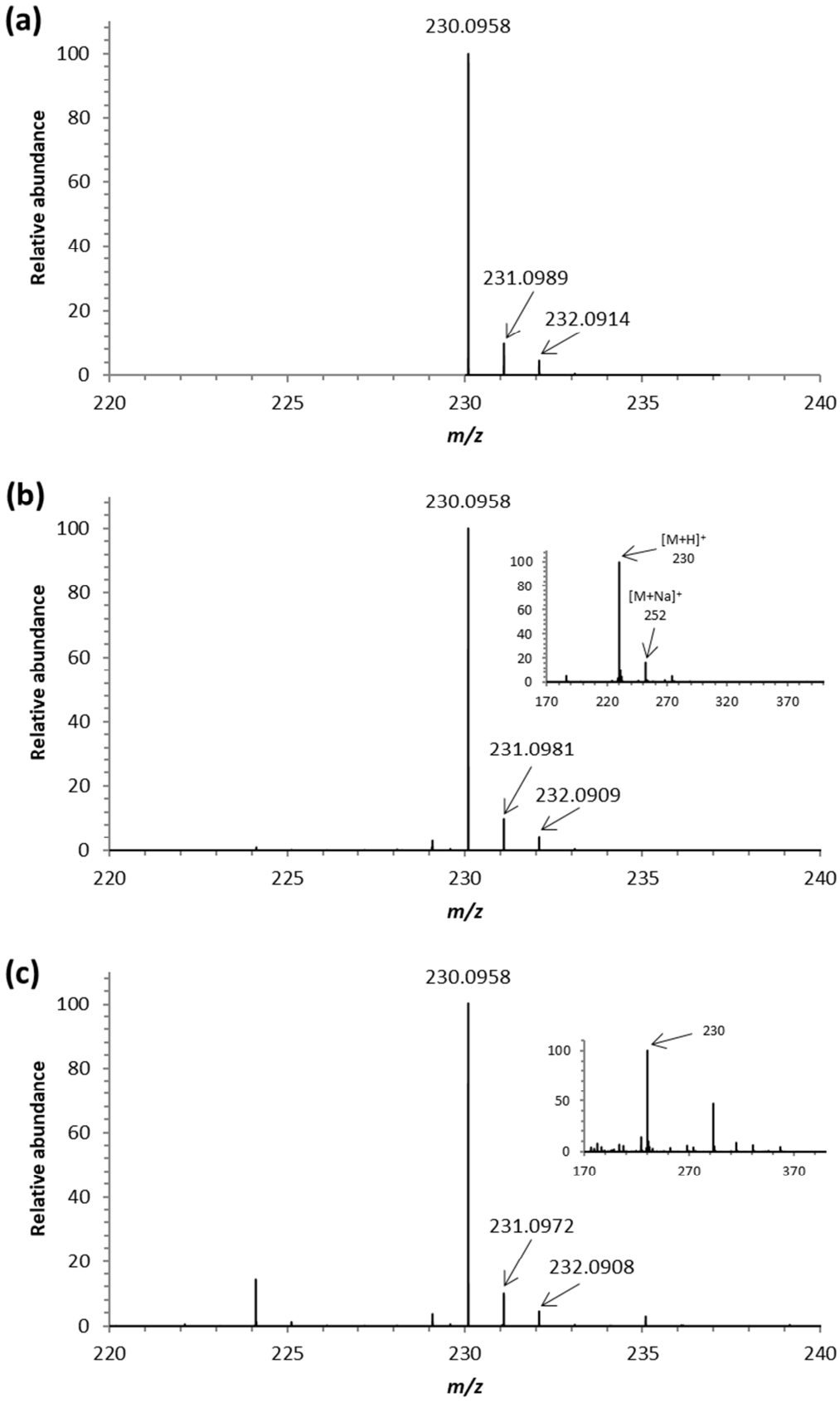

Figure S 2: HPLC/ESI-Orbitrap MS signals of a native lysate of blood cells. (a) simulated spectrum for ergothioneine, (b) background subtracted full scan spectrum of a 1 mg S L^{-1} standard solution of ergothioneine and (c) full scan spectrum of a native lysate of blood cells (volunteer 3). column: Atlantis dC18 4.6 × 150 mm; mobile phase: 20 mM ammonium formate 3% methanol pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL

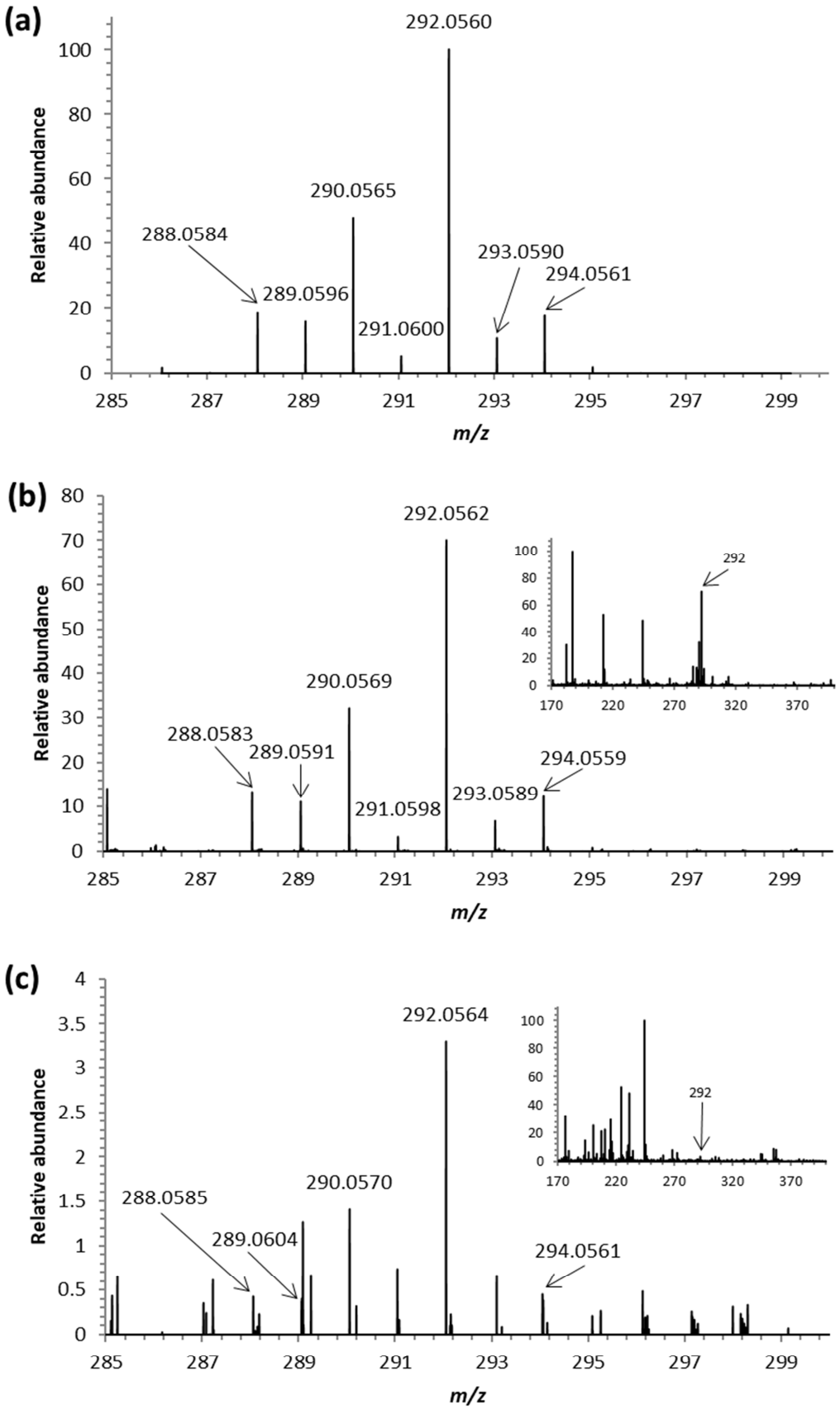

Figure S 3: HPLC/ESI-Orbitrap MS signals of a native lysate of blood cells. (a) simulated spectrum for Se-methylselenoneine, (b) background subtracted full scan spectrum of approx. 30 µg Se L^{-1} Se-methylselenoneine in a fraction collected from a water extract of fresh tuna muscle and (c) background subtracted full scan spectrum of a native lysate of blood cells (volunteer 3). column: Atlantis dC18 4.6 × 150 mm; mobile phase: 20 mM ammonium formate 3% methanol pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL

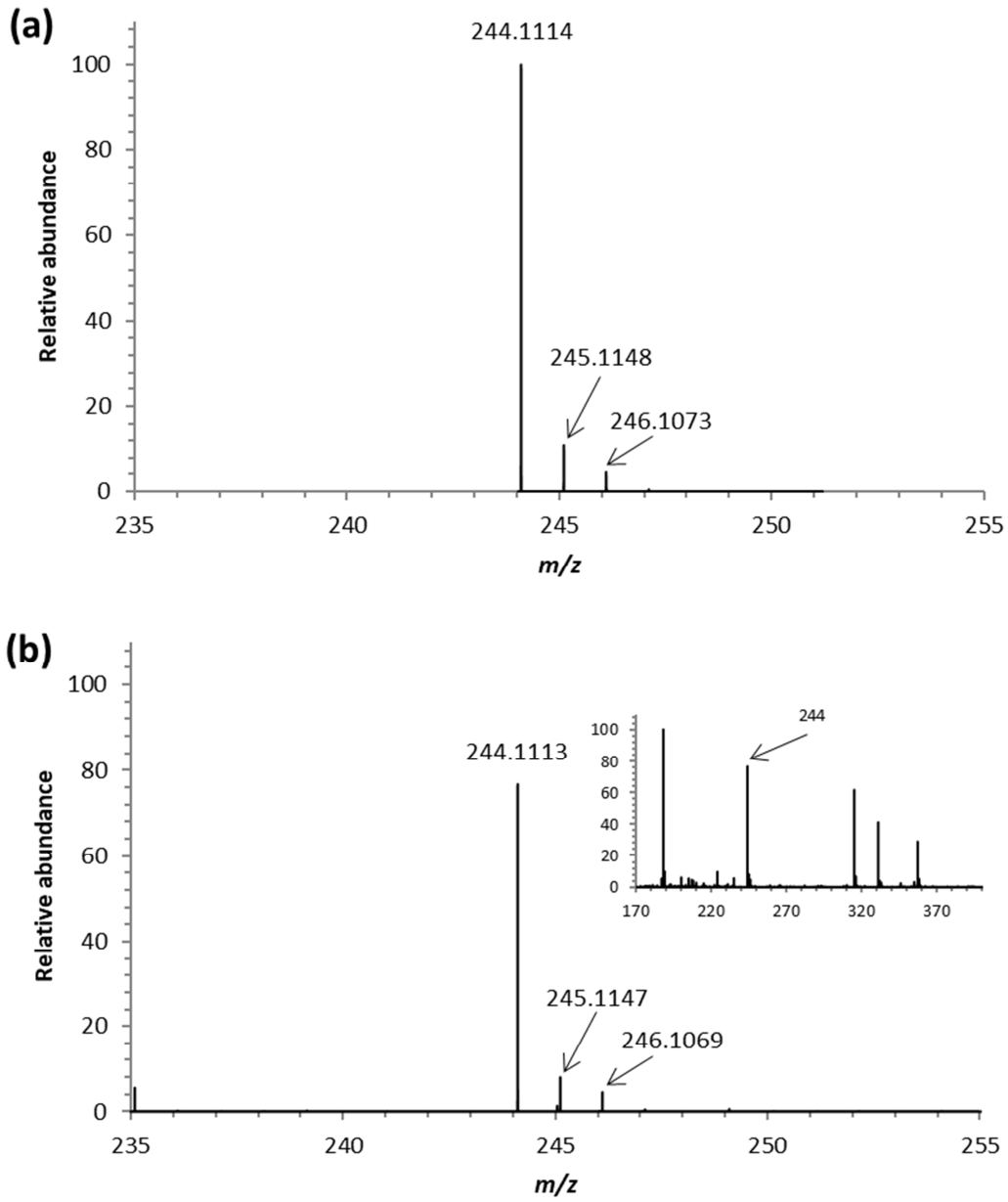

Figure S 4: HPLC/ESI-Orbitrap MS signals of a native lysate of blood cells. (a) simulated spectrum for S-methylergothioneine and (b) background subtracted full scan spectrum of a native lysate of blood cells (volunteer 3). column: Atlantis dC18 4.6 × 150 mm; mobile phase: 20 mM ammonium formate 3% methanol pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL

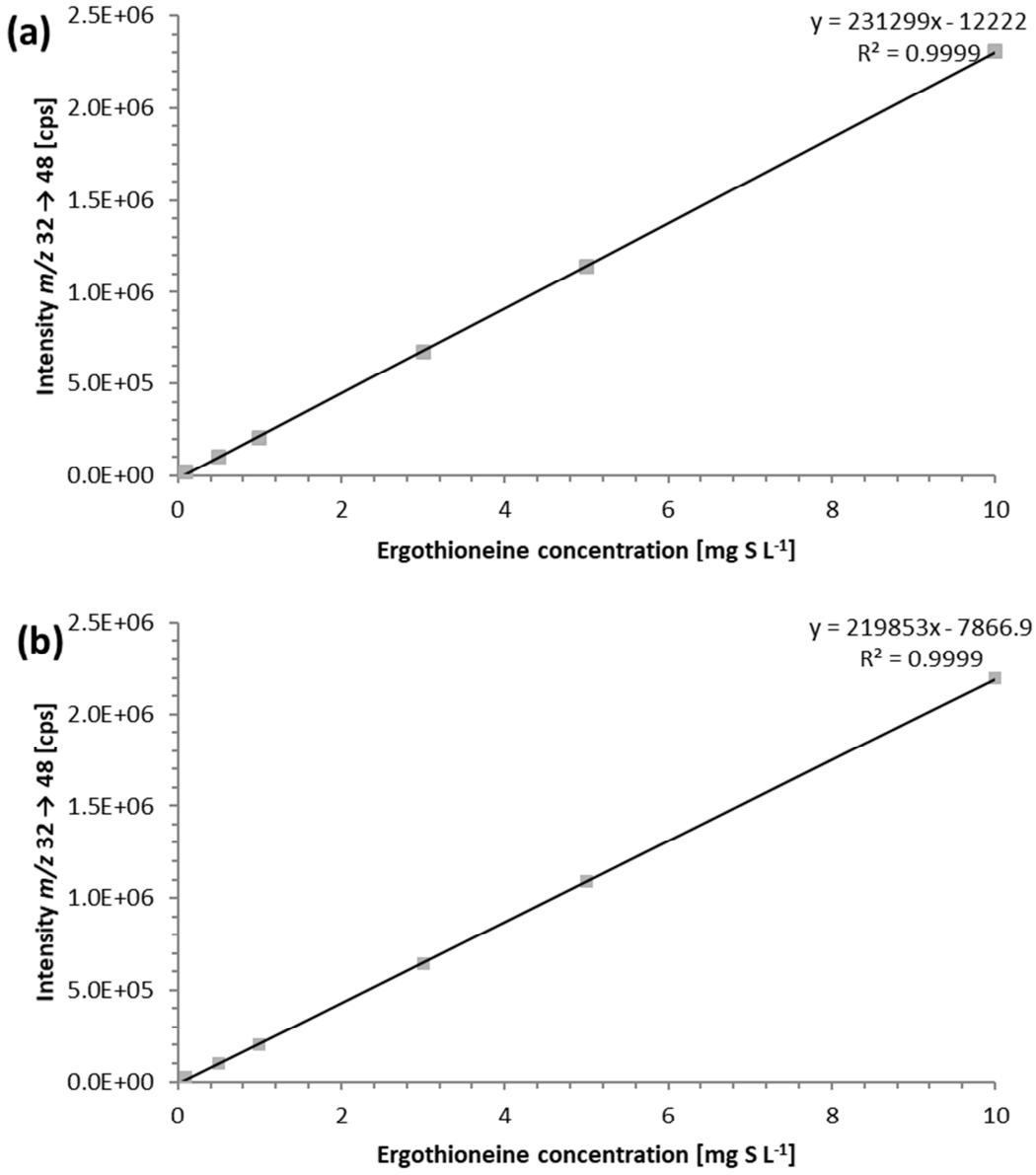

Figure S 5: Examples of external calibration plots for ergothioneine; column: Atlantis dC18 4.6 × 150 mm; mobile phase: (a) 20 mM ammonium formate 3% methanol pH 3.0 and (b) 20 mM ammonium formate 3% methanol 0.1 mM TCEP pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL

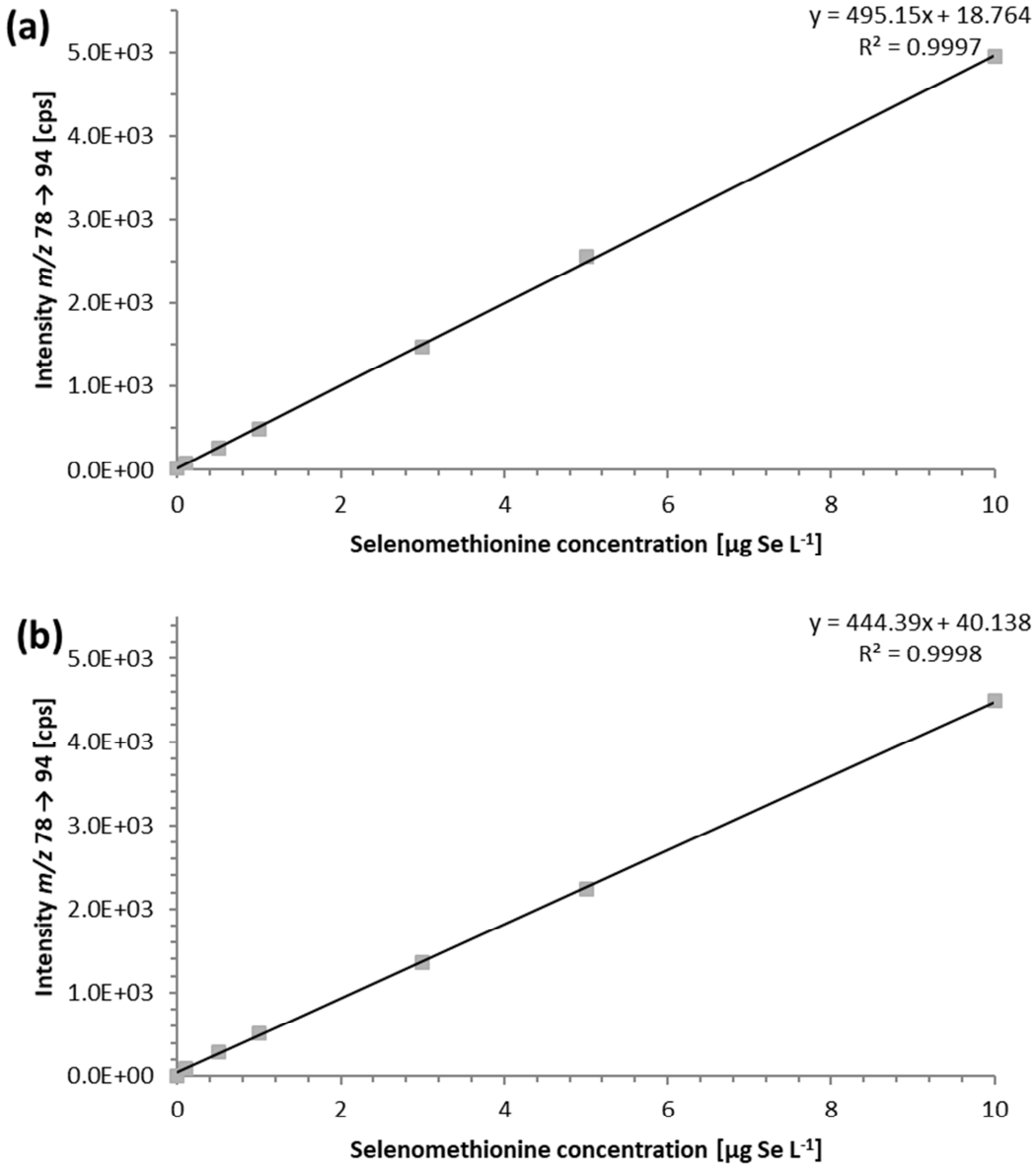

Figure S 6: Examples of external calibration plots for selenomethionine; column: Atlantis dC18 4.6 × 150 mm; mobile phase: (a) 20 mM ammonium formate 3% methanol pH 3.0 and (b) 20 mM ammonium formate 3% methanol 0.1 mM TCEP pH 3.0; flow rate: 1.0 mL min^{-1}; column temperature: 30°C; injection volume: 10 µL