Electronic Supplementary Material (ESI) for Metallomics.

Electronic Supplementary Information

Biosynthesis and Isolation of Selenoneine from Genetically Modified Fission Yeast

Nikolaus G. Turrini, Nina Kroepfl, Kenneth B. Jensen, Tamara C. Reiter, Kevin A. Francesconi, Tanja Schwerdtle, Wolfgang Kroutil and Doris Kuehnelt

Table S-1: Chromatographic conditions for selenium species determination and clean-up of selenoneine from *S. pombe* TP1803

Figure S-1: Typical HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol, flow rate: 1 mL min^{-1}, injection volume: 1 µL.

Figure S-2: Typical anion-exchange HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium; native lysate (solid line) and lysate spiked with 150 µg Se L^{-1} selenite and selenate (dashed line); insert shows the full chromatogram; Chromatograpic conditions: Column: Dionex IonPac™ AS14A-5µm RFIC™ 3 × 150 mm, column temperature: 40 °C, mobile phase: 5 mM malonate pH 9.5, flow rate: 0.7 mL min^{-1}, injection volume: 1 µL.

Figure S-3: HPLC/ICP-QQQ-MS chromatogram (oxygen mode, ca 0.3 mL O_2 min^{-1}; mass shift ^{78}Se→^{78}Se^{16}O and ^{32}S→^{32}S^{16}O) of a lysate of *S. pombe* grown in selenate-containing medium; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol, flow rate: 1 mL min^{-1}, injection volume: 1 µL.

Figure S-4: Flow injection ESI-Orbitrap-MS of fraction 2 obtained after cleanup-step 1 (NEUTRAL 1, Supplementary Material Table S1-B) of a lysate of *S. pombe* grown in selenate-containing medium directly treated with H_2O_2 before clean-up resulting in the formation of the mixed oxidised S-Se-species and oxidised ergothioneine; (a) spectrum of the mixed oxidised S-Se-species, (b) calculated isotope pattern for the mixed oxidised S-Se-species, (c) spectrum of oxidised ergothioneine, (d) calculated isotope pattern for oxidised ergothioneine; mobile phase: 3% aqueous methanol; flow rate: 0.4 mL min^{-1}, injection volume: 1 µL.

Figure S-5: HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium before (a) and after (b) rinsing of the HPLC system with 0.15% H_2O_2; Chromatographic conditions: Column: Atlantis dC18 OBT, 19 × 100 mm, column temperature: ambient, mobile phase: 3% aqueous methanol, flow rate: 5 mL min^{-1}, injection volume: 100 µL (a), 200 µL (b).

Figure S-6: HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium applying a methanol gradient; Chromatographic conditions: Column: Atlantis dC18 OBT, 19 × 100 mm, column temperature: ambient, solvent gradient from 3% aqueous methanol to 30% aqueous methanol (for gradient details see Table S-1B, Clean-up step NEUTRAL 1), flow rate: 5 mL min^{-1}, injection volume: 100 µL; vertical bars indicate the collected fraction containing oxidised selenoneine.

Figure S-7: HPLC/ICPMS chromatogram (^{78}Se (a) and ^{34}S (b)) of a lysate of *S. pombe* grown in selenate-containing medium after application of the first clean-up step (Table S-1B, Clean-up step NEUTRAL 1); Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol, flow rate: 1 mL min^{-1}, injection volume: 1 µL.

Figure S-8: HPLC/UV chromatograms (wavelength 260 nm) for monitoring of the clean-up of oxidized selenoneine from a lysate of *S. pombe* grown in selenate-containing medium using three consecutive preparative chromatographic steps (Table S1-B NEUTRAL 1 (a), ACIDIC (b), and NEUTRAL 2 (c); vertical bars indicate the collected fractions containing the oxidised selenoneine); Chromatographic conditions: Column: Atlantis dC18 OBT, 19 × 100 mm**,** column temperature: ambient, flow rate: 5 mL min^{-1}, injection volume: 1200 µL. For mobile phases, solvent gradients, and collected fractions see Table S-1B.

Figure S-9: Flow injection ESI-Orbitrap-MS of an aqueous solution of oxidised selenoneine for monitoring of the clean-up from a lysate of *S. pombe* grown in selenate-containing medium using two consecutive preparative chromatographic steps (NEUTRAL 1 (a), ACIDIC (b), Fig. 2, Supplementary Table S1-B); mobile phase: 3% aqueous methanol; flow rate: 0.4 mL min^{-1}, injection volume: 1 µL.

Figure S-10: ^{1}H-NMR spectrum (a), Heteronuclear Single Quantum Coherence Spectroscopy (b), and Heteronuclear Multiple Bond Correlation (c) of the isolated oxidised selenoneine; frequency: 500-MHz, solvent: D_2O.

Figure S-11: HPLC/ICPMS chromatograms of an aqueous solution (ca 130 µg Se L^{-1}) of the isolated oxidised selenoneine; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol (a) or 20 mM ammonium formate 3% methanol pH 3.0 (b), flow rate: 1 mL min^{-1}, injection volume: 20 µL.

Figure S-12: HPLC/Orbitrap MS chromatograms (*m/z* 553.06 and 278.04) of an aqueous solution (ca 130 µg Se L^{-1}) of the isolated oxidised selenoneine; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol (a) or 20 mM ammonium formate 3% methanol pH 3.0 (b), flow rate: 1 mL min^{-1}, injection volume: 20 µL.

Figure S-13: HPLC/ICPMS chromatogram of the isolated selenoneine; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol (a) or 20 mM ammonium formate 0.1 mM TCEP 3% methanol pH 3.0 (b), flow rate: 1 mL min^{-1}, injection volume: 20 µL.

Table S-1: Chromatographic conditions for selenium species determination and clean-up of selenoneine from *S. pombe* TP1803

A. Analytical reversed-phase HPLC			
Column	Atlantis dC18, 4.6 × 150 mm (Waters Corporation, Milford, USA) with guard column		
Column temperature	30 °C	*Flow rate*	1.0 mL min^{-1}
1. Speciation analysis of* S. pombe *cell lysates			
Mobile phase	A: water B: methanol	*Injection volume* *DAD*	1 µL 260 nm
Gradient	*Time [min]* 0 – 20 20 – 21 21 – 27 27 – 28 28 – 35		*Eluent B [%]* 3 3 – 80 80 80 – 3 3
Position of 6-port-valve between column and ICPMS	0 – 20 min 20 – 33 min 33 – 35 min	position 1; effluent from column to ICPMS position 2; effluent from column to waste position 1; effluent from column to ICPMS	
***2. Determination of isolated product from* S. pombe**			
Mobile phase	3% MeOH or 20mM ammonium formate, 3% methanol, pH 3 (adjusted with formic acid) or 20mM ammonium formate, 3% methanol, 0.1 M TCEP, pH 3 (adjusted with formic acid)	*Injection volume*	1µL or 20 µL
B. Preparative reversed-phase HPLC			
Column	Atlantis dC18 OBT, 19 × 100 mm (Waters Corporation, Milford, USA)		
Column temperature	ambient	*Injection volume*	1200 µL
Flow rate	5.0 mL min^{-1}	*DAD*	260 nm
1. Clean-up step 1 (NEUTRAL 1)			
Mobile phase	A: water B: methanol		
Gradient	*Time [min]* 0 – 20 20 – 21 21 – 30 30 – 31 31 – 45		*Eluent B [%]* 3 3 – 30 30 30 – 3 3
Fraction collection	24 – 25 min 25 – 28 min 28 – 30 min	Fraction 1 (F1); before target species Fraction 2 (F2); containing target species Fraction 3 (F3); after target species	
2. Clean-up step 2 (ACIDIC)			
Mobile phase	A: 0.05% CH_3COOH in water B: methanol		
Gradient	*Time [min]* 0 – 11 11 – 12 12 – 17 17 – 18 18 – 25		*Eluent B [%]* 2 2 – 50 50 50 – 2 2
Fraction collection	7 – 8 min 8 – 10 min 10 – 11 min	Fraction 1 (F1); before target species Fraction 2 (F2); containing target species Fraction 3 (F3); after target species	
3. Clean-up step 3 (NEUTRAL 2)			
Same settings as for clean-up step NEUTRAL 1 (see above), except for fraction collection			
Fraction collection	24 – 25 min 25 – 29 min 29 – 31 min	Fraction 1 (F1); before target species Fraction 2 (F2); containing target species Fraction 3 (F3); after target species	

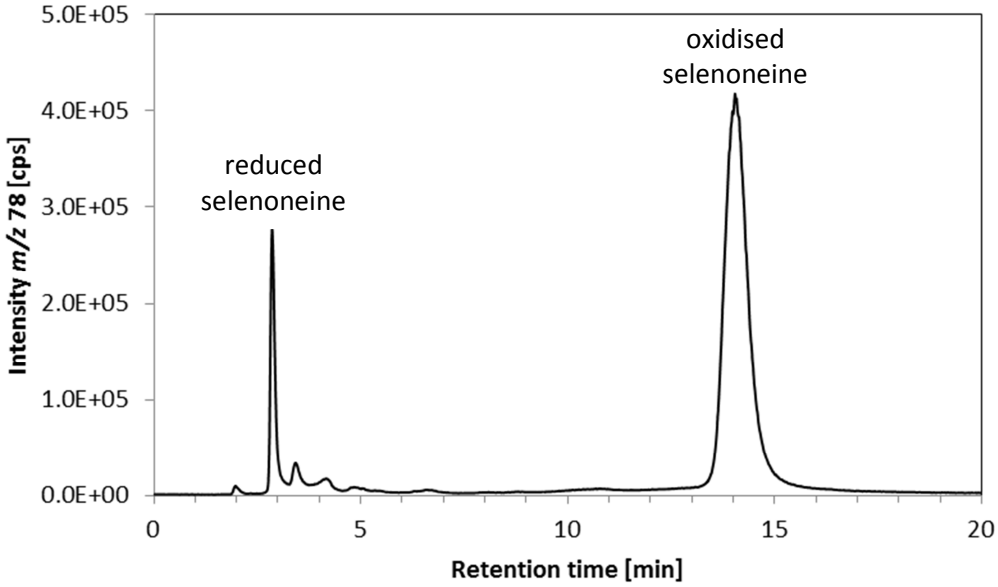


Figure S-1: Typical HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol, flow rate: 1 mL min^{-1}, injection volume: 1 µL.

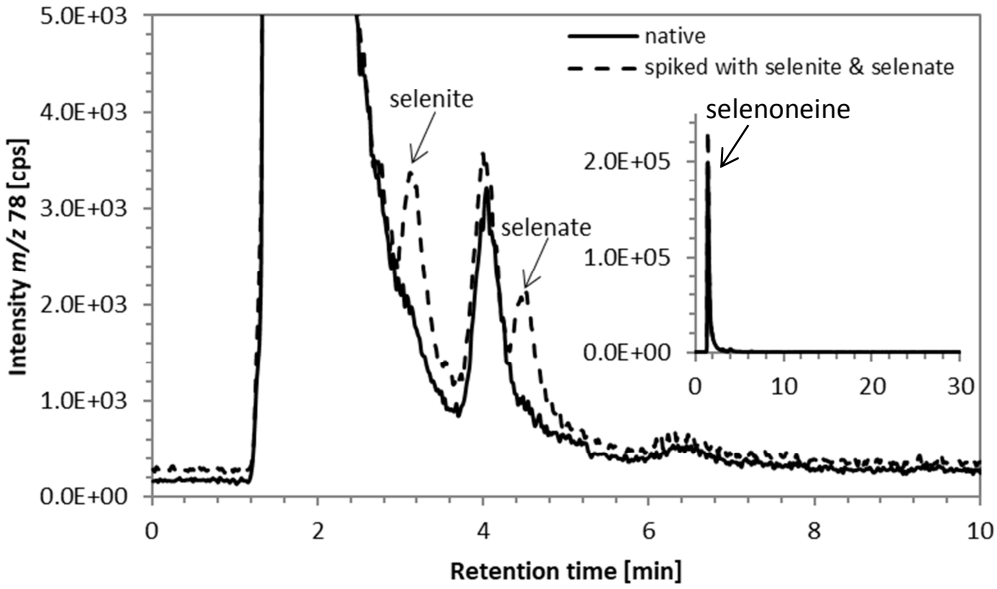


Figure S-2: Typical anion-exchange HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium; native lysate (solid line) and lysate spiked with 150 µg Se L^{-1} selenite and selenate (dashed line); insert shows the full chromatogram; Chromatograpic conditions: Column: Dionex IonPac™ AS14A-5µm RFIC™ 3 × 150 mm, column temperature: 40 °C, mobile phase: 5 mM malonate pH 9.5, flow rate: 0.7 mL min^{-1}, injection volume: 1 µL.

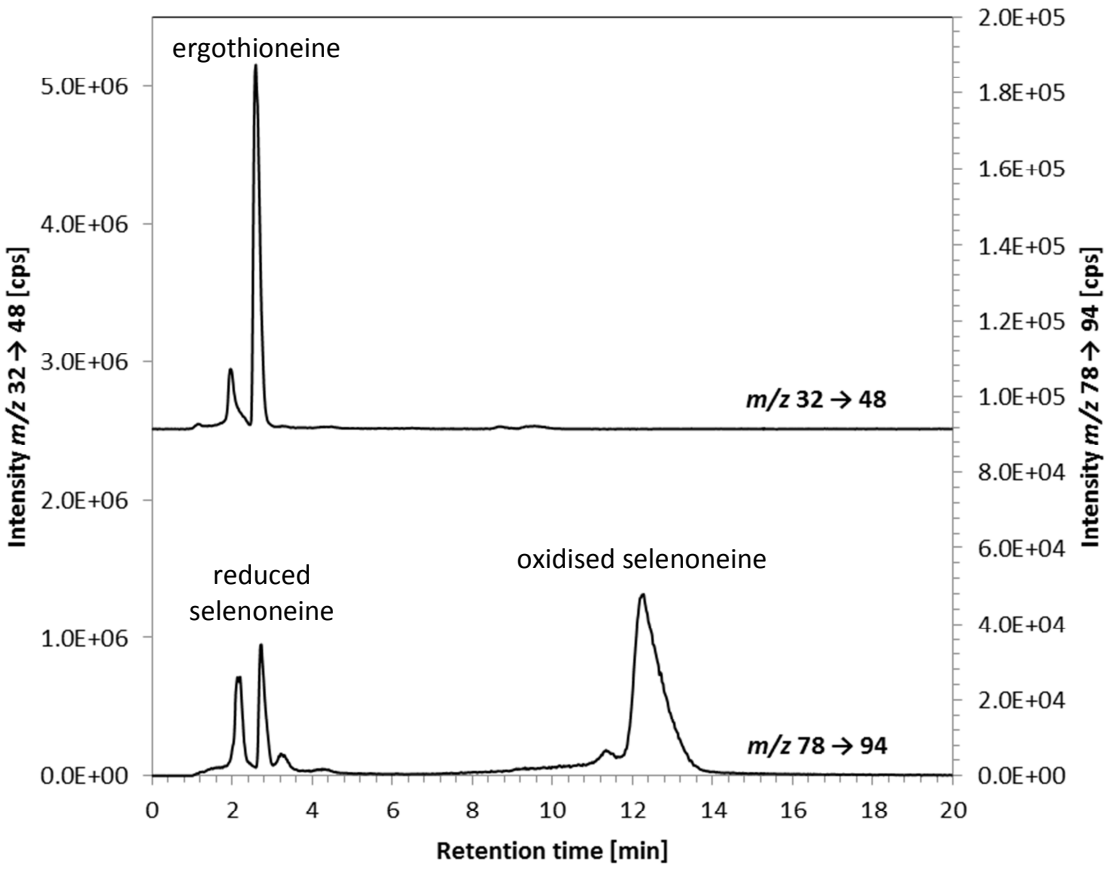


Figure S-3: HPLC/ICP-QQQ-MS chromatogram (oxygen mode, ca 0.3 mL O_2 min^{-1}; mass shift $^{78}Se \rightarrow {}^{78}Se^{16}O$ and $^{32}S \rightarrow {}^{32}S^{16}O$) of a lysate of *S. pombe* grown in selenate-containing medium; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol, flow rate: 1 mL min^{-1}, injection volume: 1 µL.

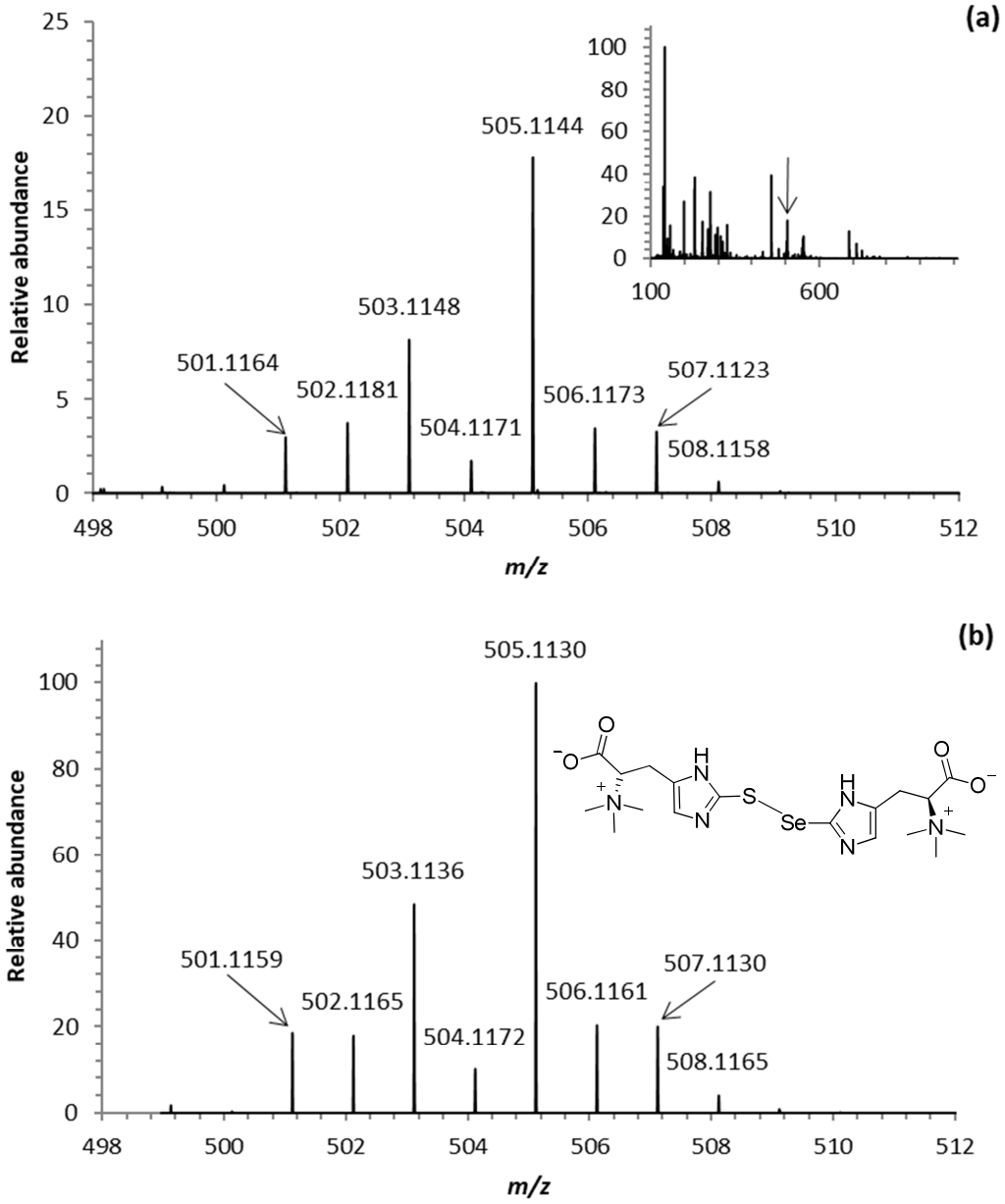
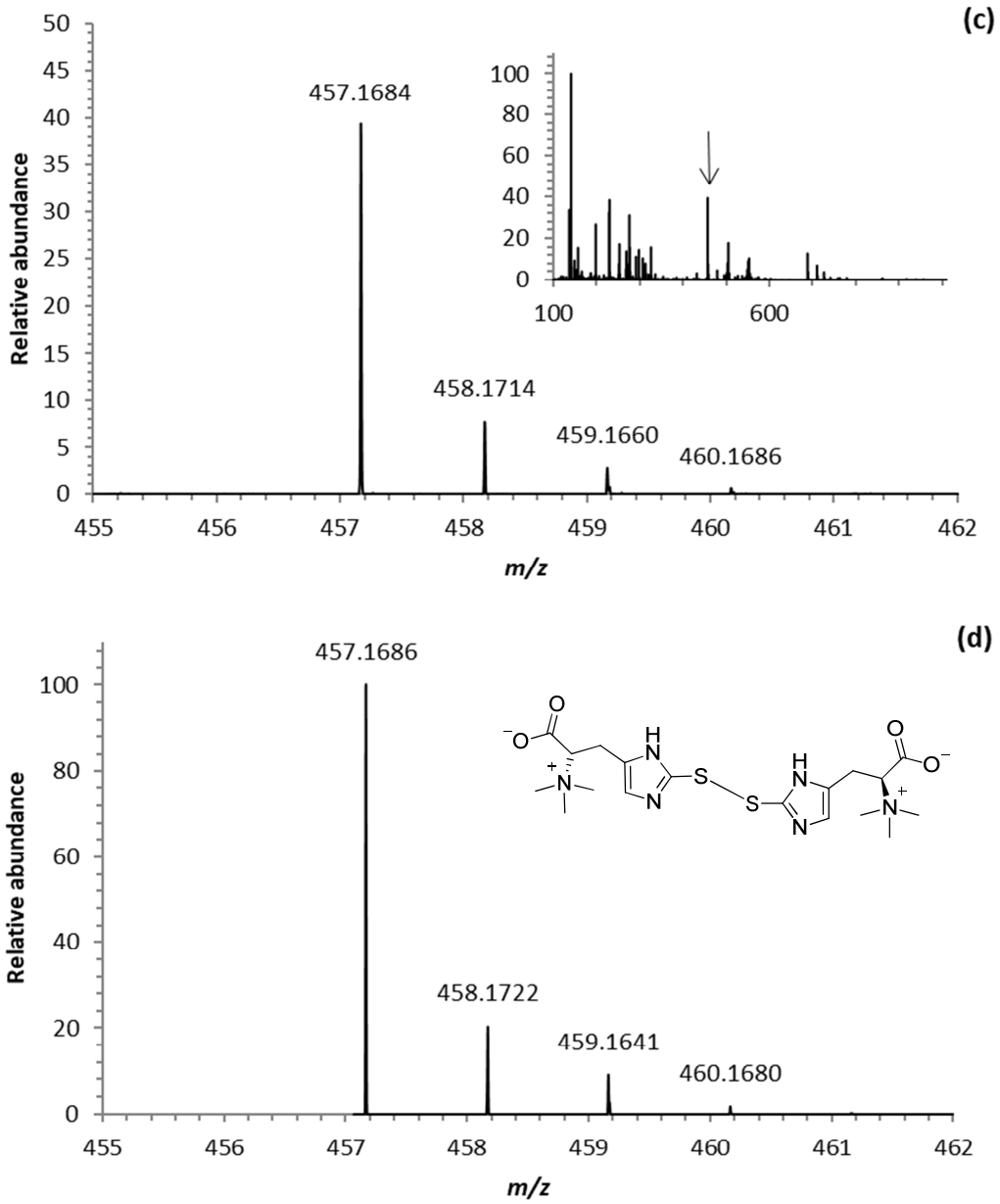


Figure S-4: Flow injection ESI-Orbitrap-MS of fraction 2 after cleanup-step 1 (NEUTRAL 1, Supplementary Material Table S1-B) of a lysate of *S. pombe* grown in selenate-containing medium and treated by direct addition of H_2O_2 to the lysate; (a) spectrum of the mixed oxidised S-Se-species, (b) calculated isotope pattern for the mixed oxidised S-Se-species; mobile phase: 3% aqueous methanol; flow rate: 0.4 mL min^{-1}, injection volume: 1 µL.



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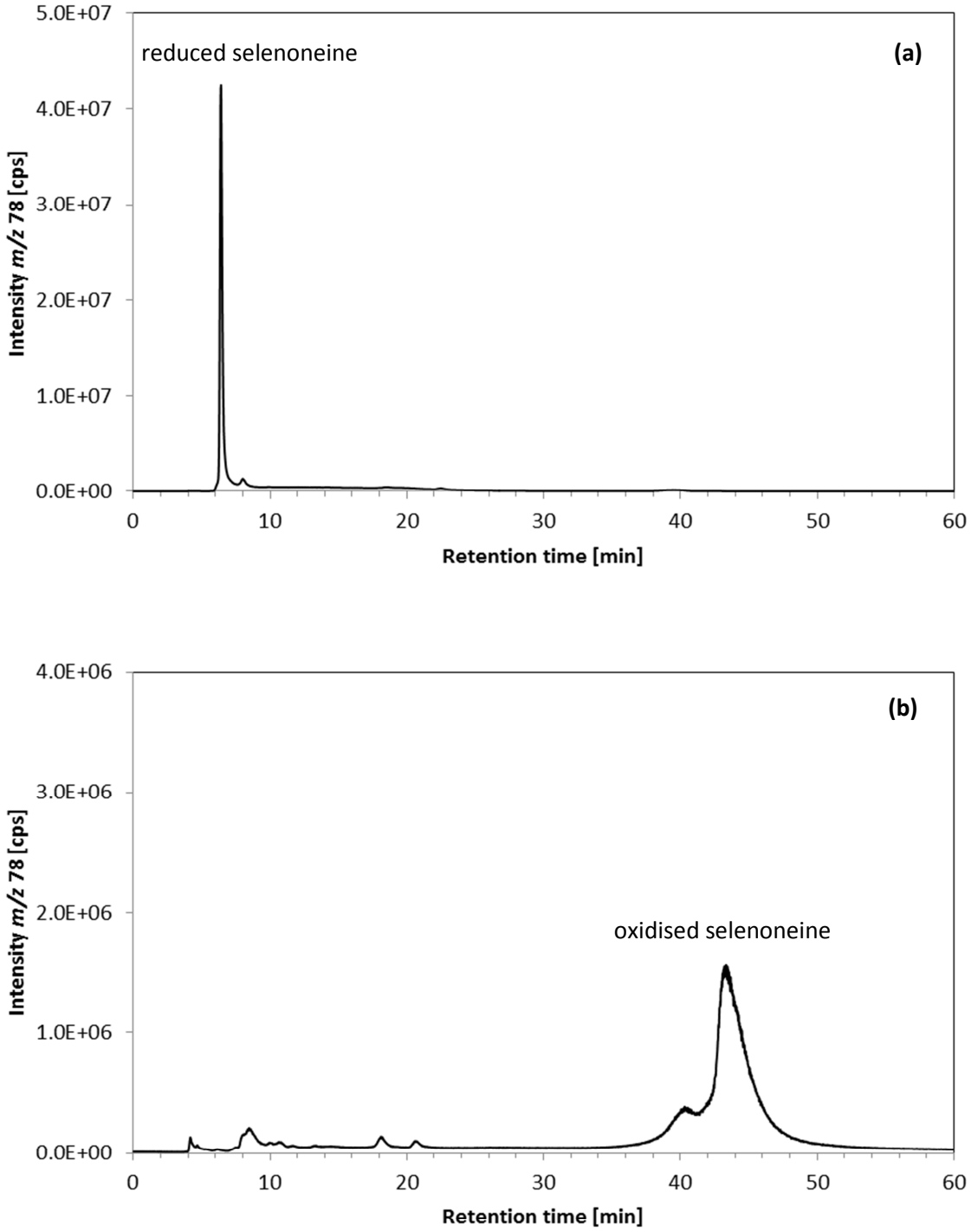
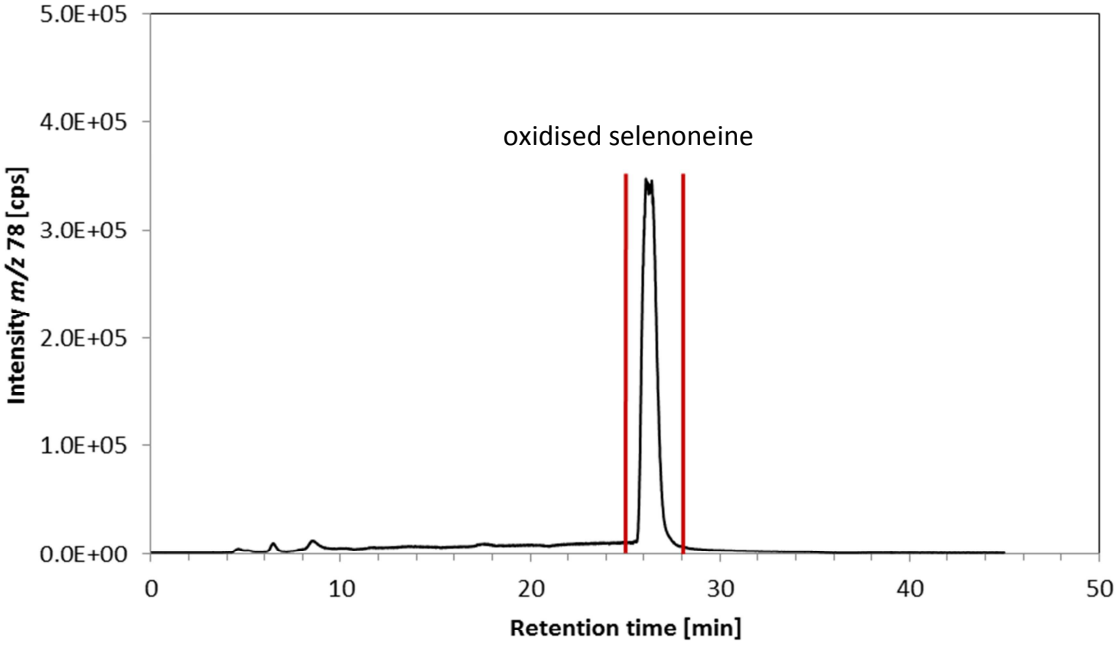


Figure S-5: HPLC/ICPMS chromatogram (^{78}Se) of a lysate of *S. pombe* grown in selenate-containing medium before (a) and after (b) rinsing of the HPLC system with 0.3% H_2O_2; Chromatographic conditions: Column: Atlantis dC18 OBT, 19 × 100 mm, column temperature: ambient, mobile phase: 3% aqueous methanol, flow rate: 5 mL min^{-1}, injection volume: 100 µL (a), 200 µL (b).



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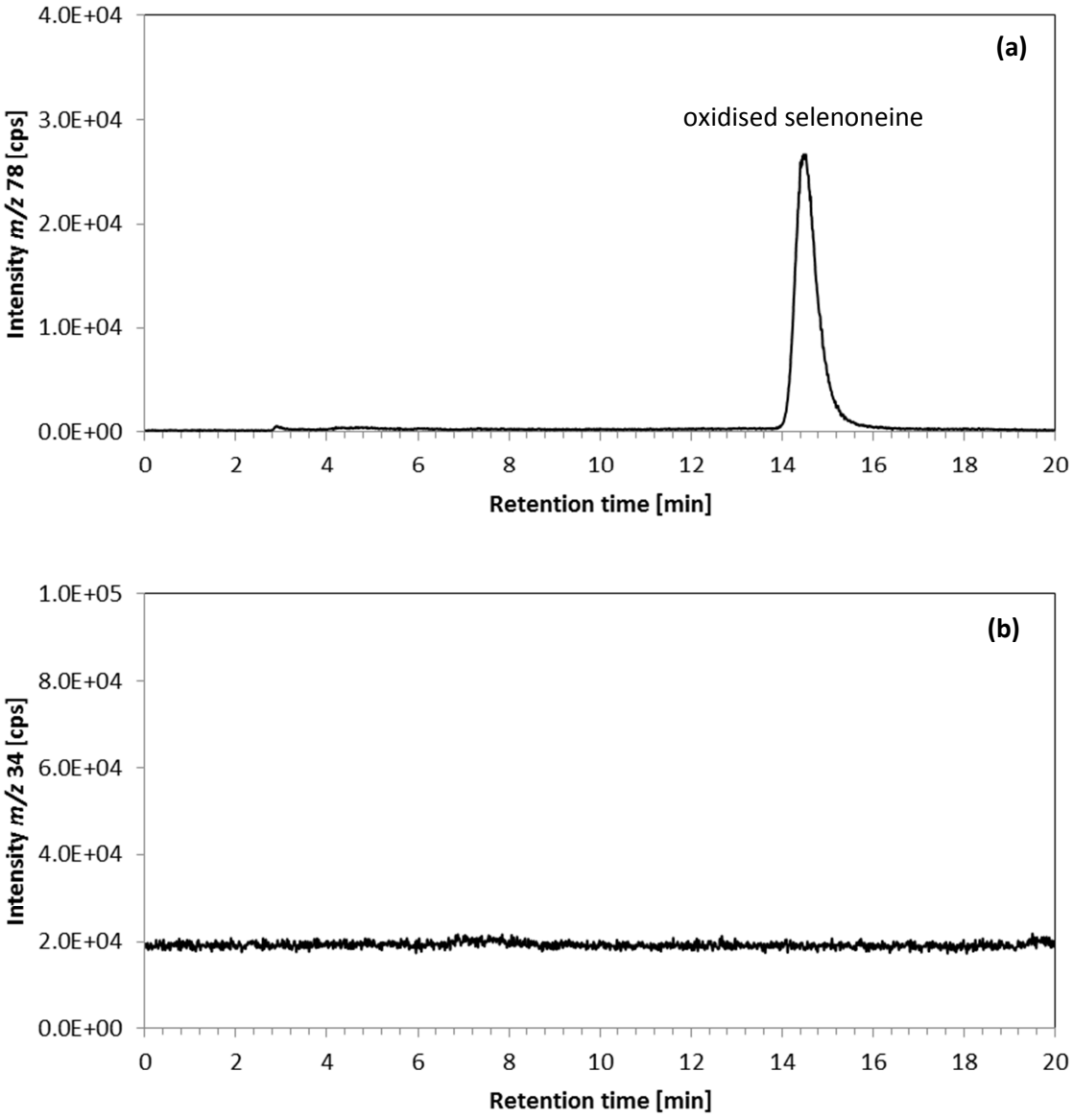

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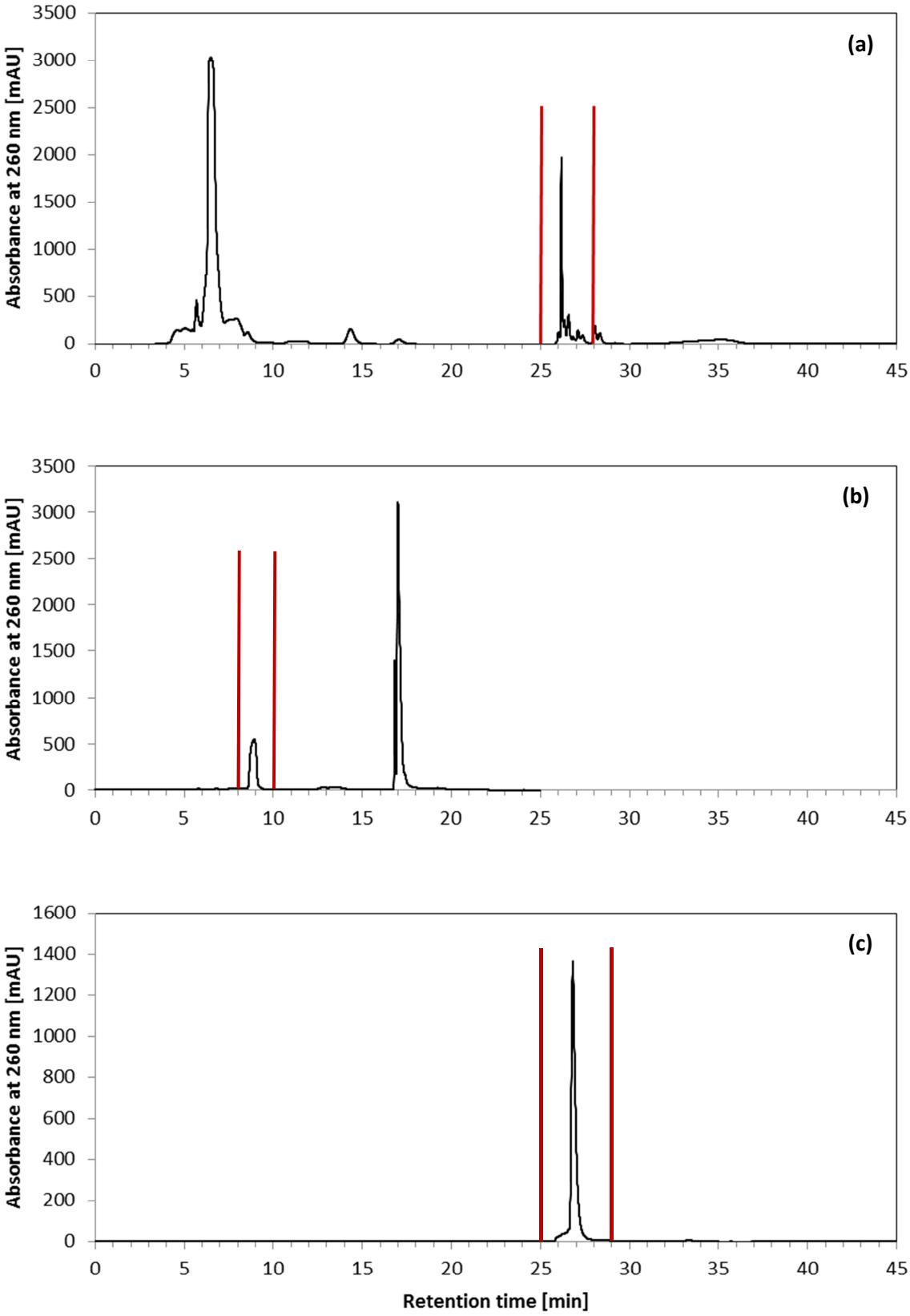


Figure S-8: HPLC/UV chromatograms (wavelength 260 nm) for monitoring of the clean-up of oxidised selenoneine from a lysate of *S. pombe* grown in selenate-containing medium using three consecutive preparative chromatographic steps (Table S1-B NEUTRAL 1 (a), ACIDIC (b), and NEUTRAL 2 (c); vertical bars indicate the collected fractions containing the oxidised selenoneine); Chromatographic conditions: Column: Atlantis dC18 OBT, 19 × 100 mm, column temperature: ambient, flow rate: 5 mL min^{-1}, injection volume: 1200 µL. For mobile phases, solvent gradients, and collected fractions see Table S-1B.

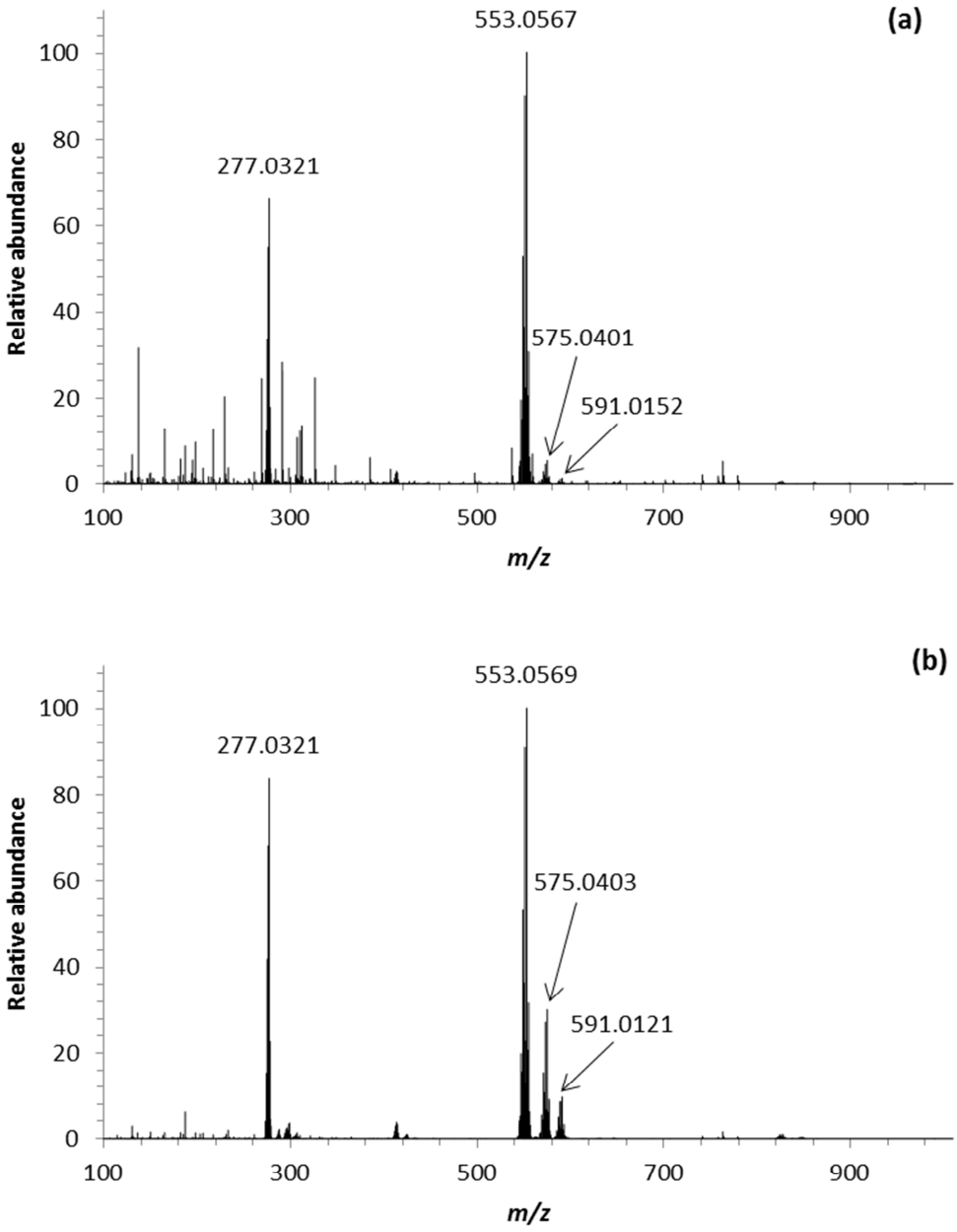


Figure S-9: Flow injection ESI-Orbitrap-MS of an aqueous solution of oxidised selenoneine for monitoring of the clean-up from a lysate of *S. pombe* grown in selenate-containing medium using two consecutive preparative chromatographic steps (NEUTRAL 1 (a), ACIDIC (b), Fig. 2, Supplementary Table S1-B); mobile phase: 3% aqueous methanol; flow rate: 0.4 mL min^{-1}, injection volume: 1 µL.

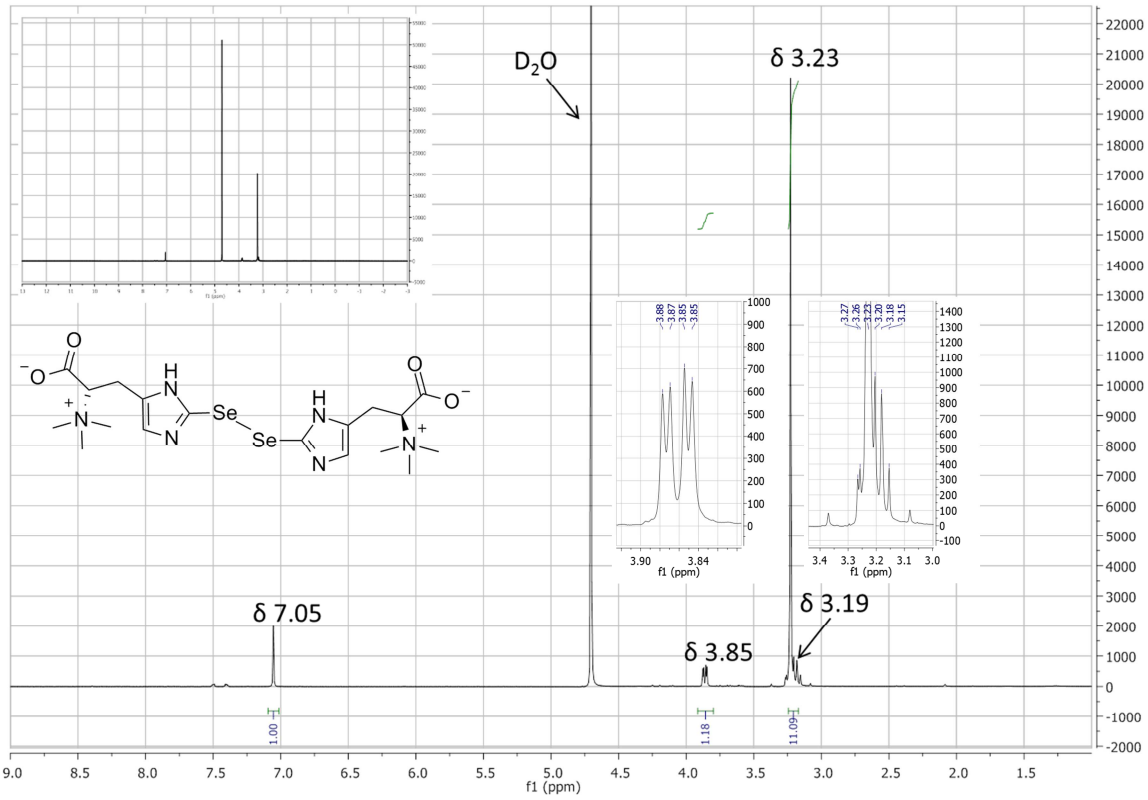


Figure S-10(a): ^{1}H-NMR spectrum of the isolated oxidised selenoneine; frequency: 500-MHz, solvent: D_2O.

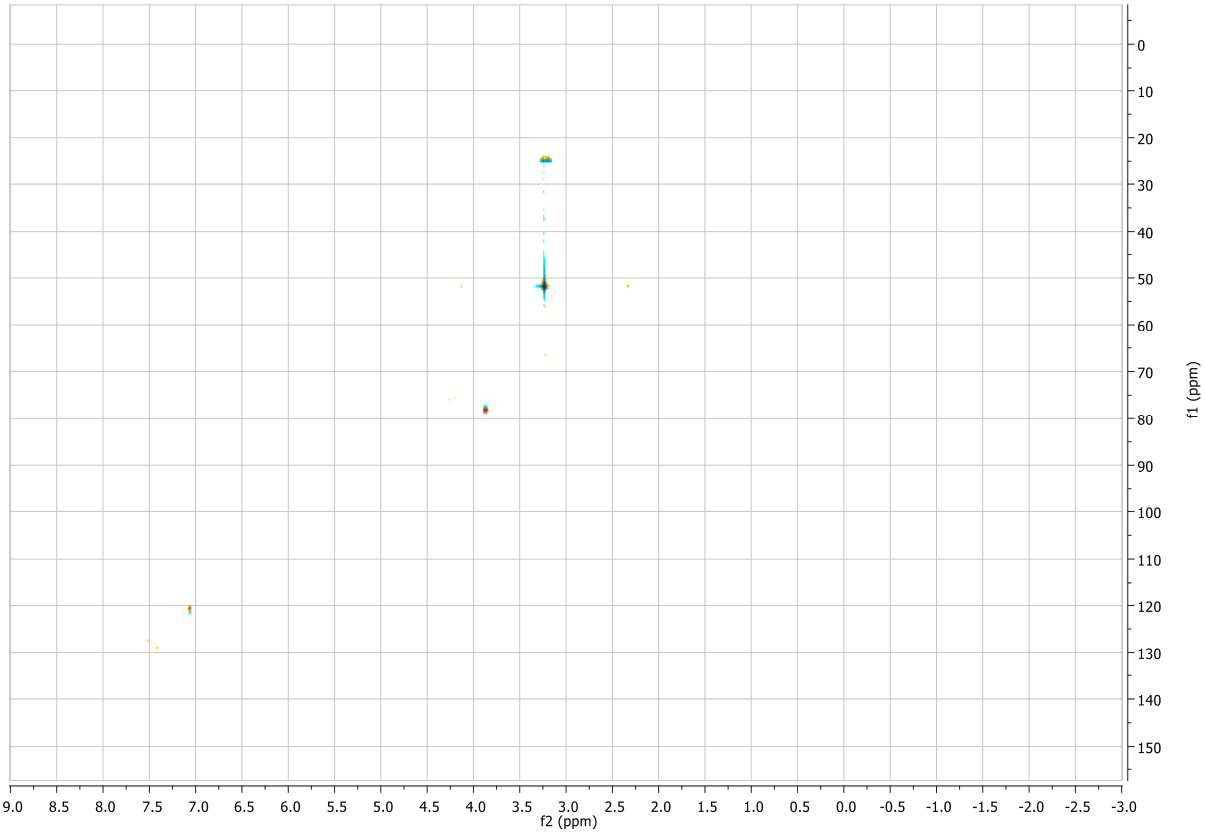


Figure S-10(b): Heteronuclear Single Quantum Coherence Spectroscopy of the isolated oxidised selenoneine; frequency: 500-MHz, solvent: D_2O.

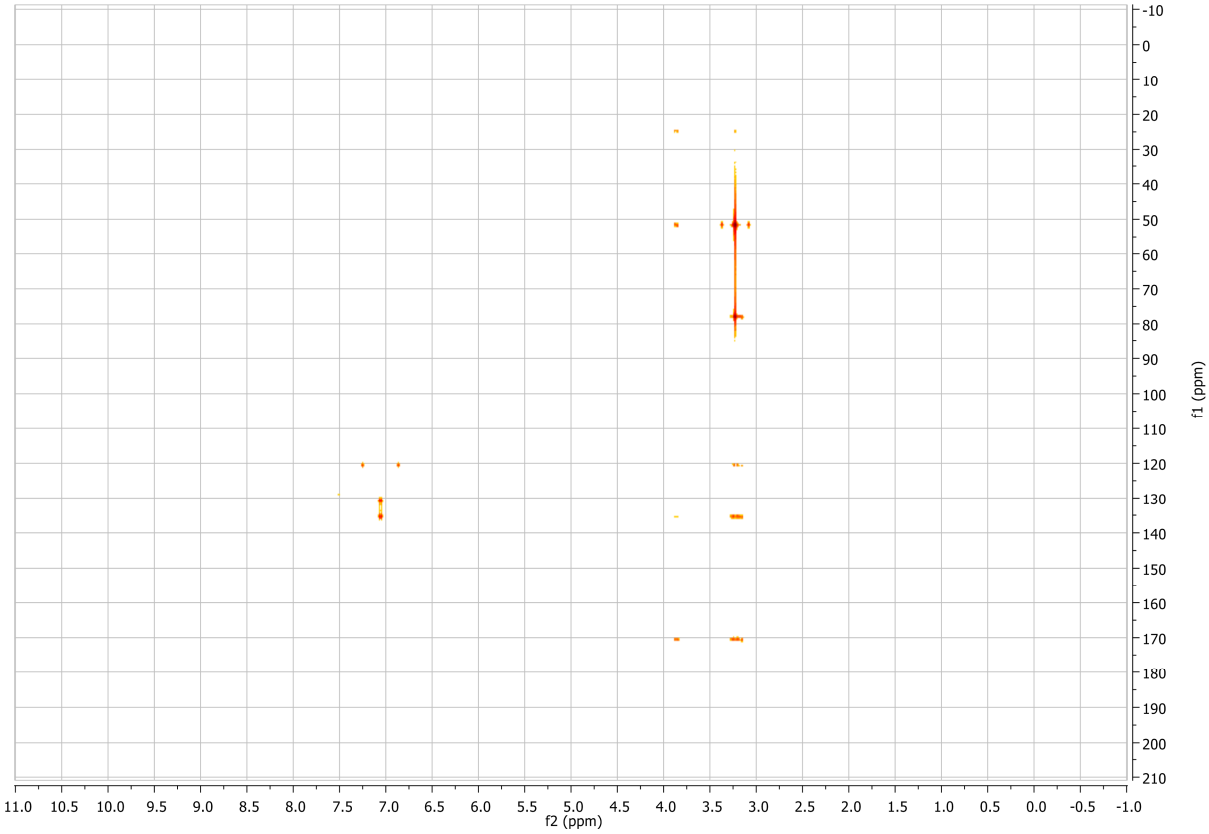
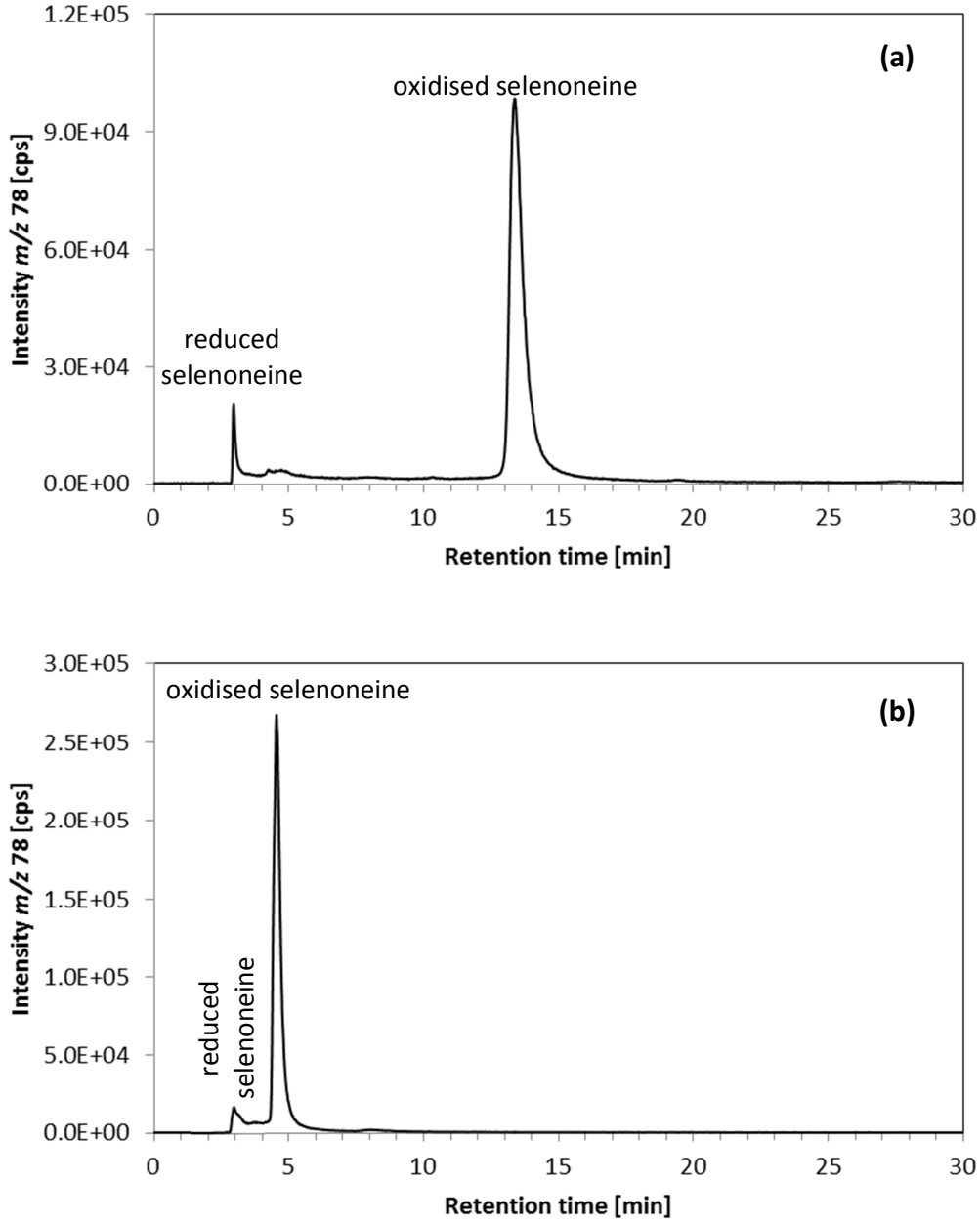


Figure S-10(c): Heteronuclear Multiple Bond Correlation of the isolated oxidised selenoneine; frequency: 500-MHz, solvent: D_2O.



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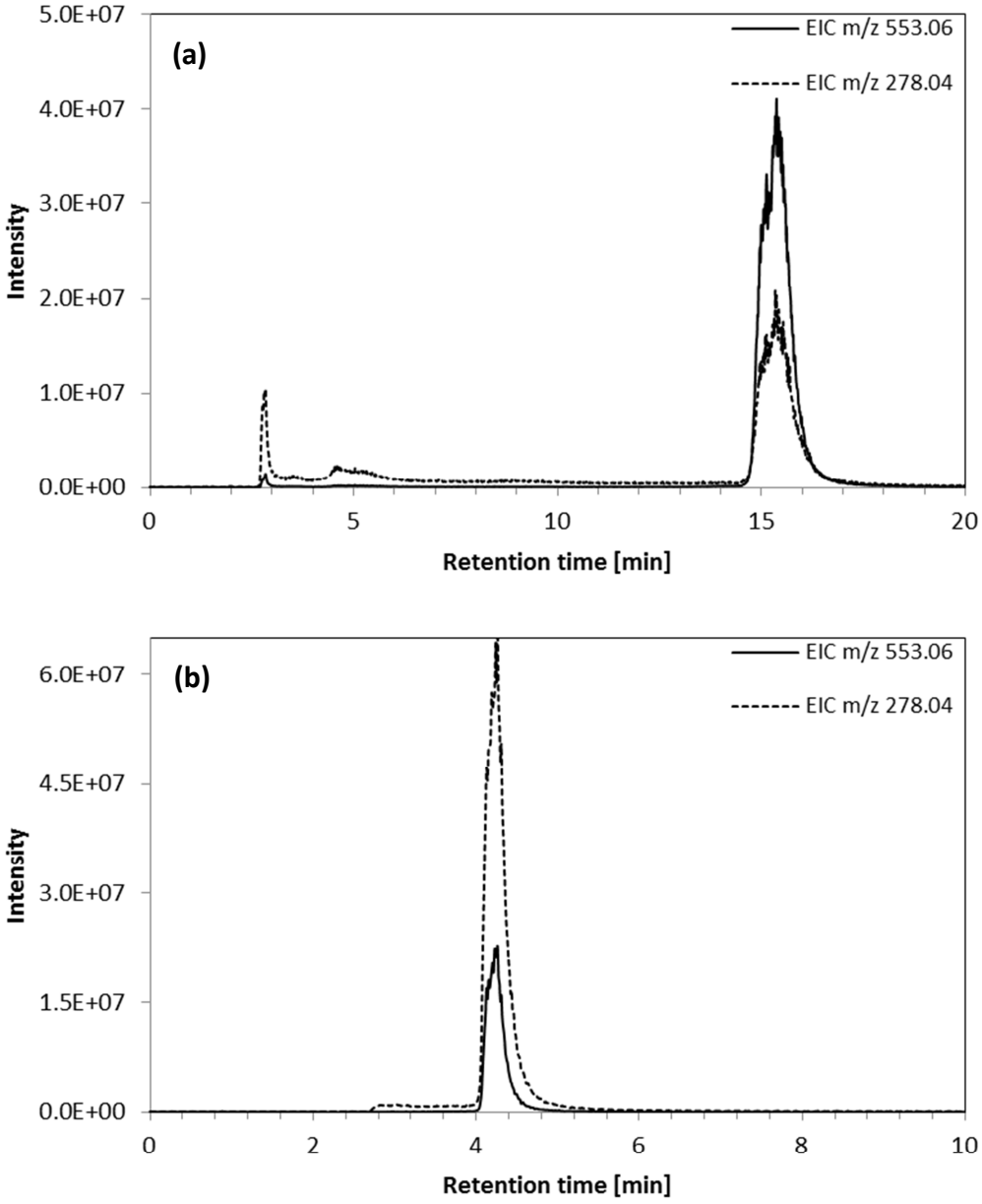

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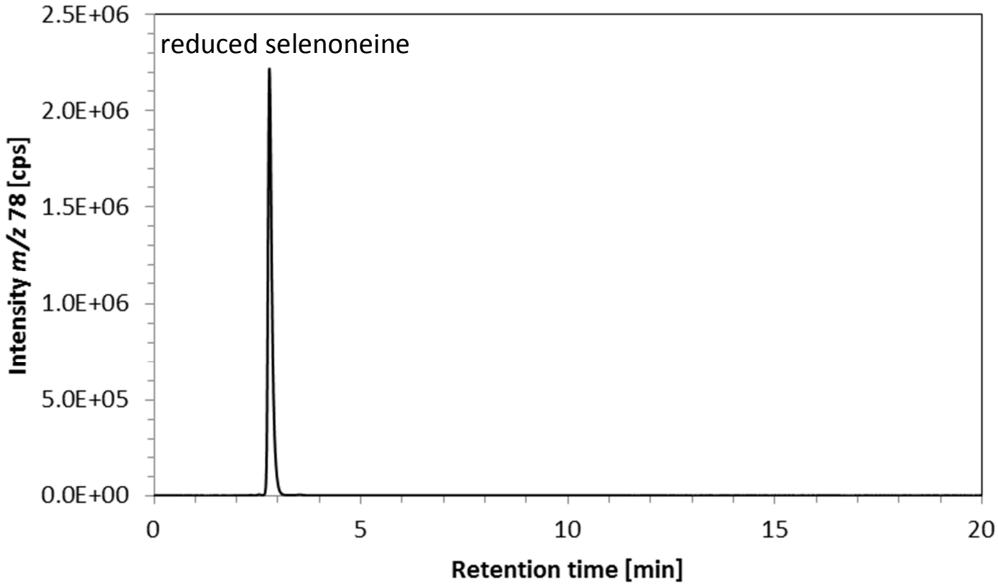

Figure S-13: HPLC/ICPMS chromatogram of the isolated selenoneine; Chromatographic conditions: Column: Atlantis dC18, 4.6 × 150 mm, column temperature: 30 °C, mobile phase: 3% aqueous methanol (A) or 20 mM ammonium formate 0.1 mM TCEP 3% methanol pH 3.0 (B), flow rate: 1 mL min^{-1}, injection volume: 20 µL.