Supplementary Material

A general covalent binding model between cytotoxic selenocompounds and albumin revealed by mass spectrometry and X-ray absorption spectroscopy

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Supplementary Table 1. LC-MS parameters to measure parent selenocompound.

	Item	$CysSe_2$	MeSeA	Ebselen	p-XSC	MeSeCys	SeMet
LC	Column	YMC AQ12S05-1546WT					
	Mobile Phase	ACN/H_2O with 0.1% Formic Acid					
	Flow Rate (ml/min)	0.6	0.4	0.4	0.2	0.4	0.4
	ACN Volume (%)	70%	70%	70%	70%	30%	20%
	Running Time (min)	5	6	8	15	6	7
MS	Spray Voltage (V)	4500	5000	5000	5000	5000	5000
	Sheath Gas (Arb)	50	10	40	50	30	60
	Auxiliary Gas (Arb)	5	5	5	5	15	5
	Ion Sweep Gas (Arb)	1	2	2	2	2	2
	Skimmer Offset (V)	-17	-18	-2	-15	-24	-16
	Capillary Temperature (°C)	400	297	400	375	312	400
	Tube Lens Offset (V)	100	131	106	100	128	86
	Collision Energy	1.2	N.A.	N.A.	N.A.	N.A.	N.A.
	Collision Pressure (mTorr)	-13	N.A.	N.A.	N.A.	N.A.	N.A.
	Ion of Monitor (m/z)	336.8→247.8	128.9	275.9	209.9	166.9	197.9

Arb: Arbitrary units. N.A.: Not applicable.

Supplementary Table 2. LC-MS parameters to measure selenol derivative.

	Item	CysSe-NEM	MeSeA-NEM	Ebselen-NEM	p-XSC-NEM
LC	Column	YMC AQ12S05-1546WT			
	Mobile Phase	ACN/H_2O with 0.1% Formic Acid			
	Flow Rate (ml/min)	0.4	0.2	0.4	0.2
	ACN Volume (%)	20	70	70	70
	Running Time (min)	11	15	10	20
MS	Spray Voltage (V)	4500	5000	4000	5000
	Sheath Gas (Arb*)	50	40	40	40
	Auxiliary Gas (Arb)	5	35	5	50
	Ion Sweep Gas (Arb)	1	1	2	1.5
	Skimmer Offset (V)	-17	-6	-15	-15
	Capillary Temperature (°C)	400	400	400	277
	Tube Lens Offset (V)	100	80	75	186
	Ion of Monitor (m/z)	294.9	221.9	402.9	309.9

Supplementary Table 3. Linear combination fitting results of X-ray absorption near edge spectra. The fitting was performed over the spectra for selenocompound-human plasma mixture between -20 and 50 eV keeping E_0 fixed and equal to 0 eV.

SeC	Total SeC (μg/ml)	SeC (%)	SeC-HSA (%)	R-factor	Chi-square	Reduced Chi-squre
$CysSe_2$	20	19.6	80.4	0.002	0.050	0.0004
MeSeA	20	0	100.0	0.029	0.093	0.0068
Ebselen	20	8.5	91.5	0.004	0.103	0.0009
p-XSC	20	0	100.0	0.005	0.164	0.0012

Supplementary Table 4. Stoichiometry of selenocompound versus albumin thiol. The selenol intermediate of p-XSC has two selenol groups, but it was unclear whether both could bind to albumin thiol at the same time.

SeC	Stoichiometry (SeC: Selenol intermediate: Albumin thiol)
$CysSe_2$	1:2:2
MeSeA	1:1:1
Ebselen	1:1:1
p-XSC	1:1:1 or 1:1:2

Supplementary Table 5. Summary of linearity and limit of detection. SeC-spiked plasma was analyzed using RECID. Linearity was evaluated by considering the detector response (peak area) to different amounts of SeC by means of linear regression. Results were generated from three technical replicates. Limit of detection (LOD) was defined as signal/noise ratio equal to 3.

SeC	Linear range (µg/mL)	Slope (Mean ± SD)	Y-Intercept (Mean ± SD)	Correlation coefficient	LOD (µg/mL)
$CysSe_2$	2-40	67940 ± 928.7	27250 ± 19830	0.9990	0.5
MeSeA	1-40	96420 ± 803.1	30830 ± 16560	0.9997	0.2
Ebselen	1-40	131100 ± 1941	-34530 ± 40020	0.9980	0.2

Supplementary Table 6. Validation of RECID. SeC-spiked plasma was processed using RECID. Precision was shown as the relative standard deviation of three individual measures of the analyte in multiple aliquots of the same stock solution. Accuracy was the percentage of concentration calculated with calibration curve to the nominal concentration. Recovery was the percentage of peak area of the analyte in plasma to that in solution. Results about accuracy and recovery are shown as the mean ± SD of three technical replicates.

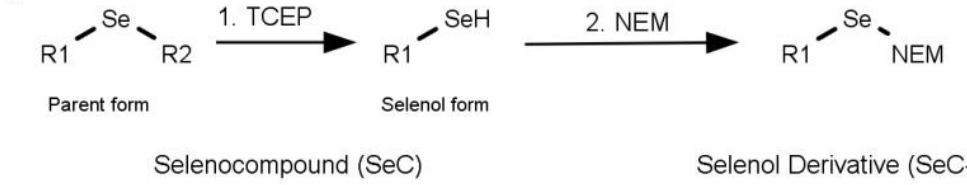
SeC		Precision (%)		Accuracy (%)	Recovery (%)
Type	Level (μg/mL)	Intra-day	Inter-day		
$CysSe_2$	2	1.7	4.4	90.3 ± 1.8	88.7 ± 3.9
	10	7.7	7.3	108.9 ± 8.8	78.6 ± 4.0
	40	5.5	3.7	101.5 ± 5.7	79.0 ± 3.8
MeSeA	1	5.6	0.9	115.3 ± 7.9	100.5 ± 0.7
	10	5.5	6.7	97.3 ± 5.7	86.5 ± 1.5
	40	2.6	1.4	103.0 ± 2.6	89.9 ± 0.3
Ebselen	1	3.7	2.2	107.0 ± 2.9	79.9 ± 6.9
	10	0.5	0.8	107.8 ± 2.5	75.3 ± 2.7
	40	3.4	3.8	103.2 ± 3.5	71.5 ± 2.0

Supplementary Table 7. Stability of selenocompound in plasma and selenol derivative in processed sample. SeC-spiked plasma was treated as indicated and then analyzed using RECID. SeC-NEM in processed sample was treated in the same way and then subjected to LC-MS analysis. Stability was shown as the percentage of the peak area after indicated treatment to that from base line value. SeC concentrations were 20 μg/mL. Results are shown as the mean ± SD of three technical replicates.

SeC	Sample Type	Treatment			
		RT/24 hr	4 °C/24 hr	-20 °C/24 hr	Freeze-Thaw/5 Cycles
$CysSe_2$	Spiked Plasma	98.6 ± 7.1	100.9 ± 7.9	99.6 ± 6.4	102.2 ± 0.6
	Processed	104.3 ± 0.4	99.1 ± 1.8	101.5 ± 4.6	97.1 ± 1.9
MeSeA	Spiked Plasma	110.9 ± 2.0	108.9 ± 4.8	116.0 ± 3.2	117.3 ± 0.5
	Processed	114.3 ± 0.3	106.7 ± 0.5	103.2 ± 1.0	104.3 ± 0.4
Ebselen	Spiked Plasma	110.3 ± 1.8	113.1 ± 0.5	108.2 ± 3.2	97.2 ± 2.7
	Processed	106.8 ± 3.0	106.7 ± 0.3	108.7 ± 0.7	104.5 ± 0.6

Supplementary Figure 1. Synthesis of selenol derivative. (a) Scheme for synthesis of selenol derivative (SeC-NEM). (b) Formulation for SeC-NEM synthesis.

a

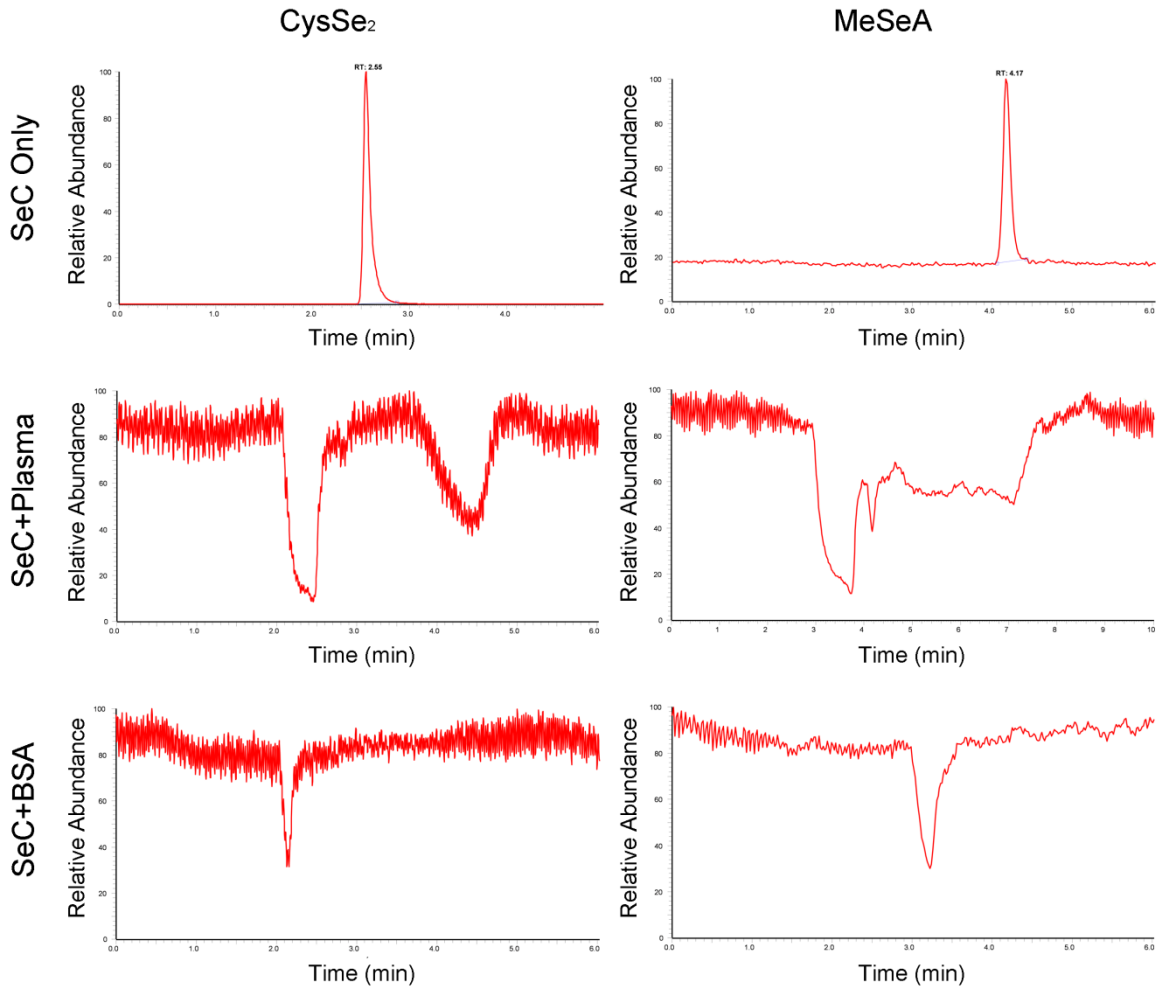


Selenocompound (SeC) Selenol Derivative (SeC-NEM)

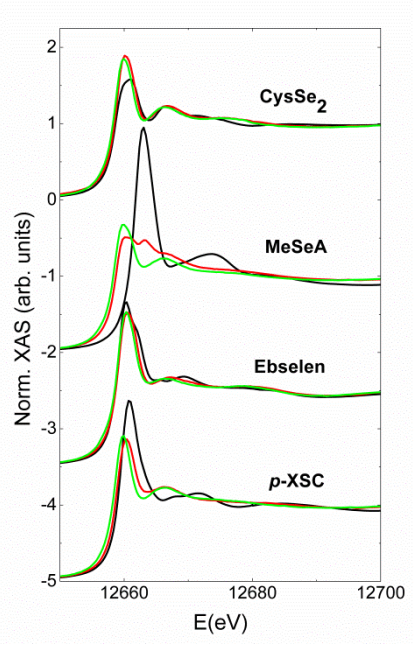
b

Selenocompound				NEM (20 mg/ml in ACN)	TCEP (50 mg/ml in H_2O)
Type	Solvent	Concentration	Amount		
$CysSe_2$	H_2O; Neutralized	10 mg/ml	2 ml	1 ml	0.46 ml
MeSeA	H_2O; Neutralized	11 mg/ml	2 ml	1.5 ml	1.1 ml
Ebselen	ACN	14 mg/ml	1 ml	0.7 ml	0.34 ml

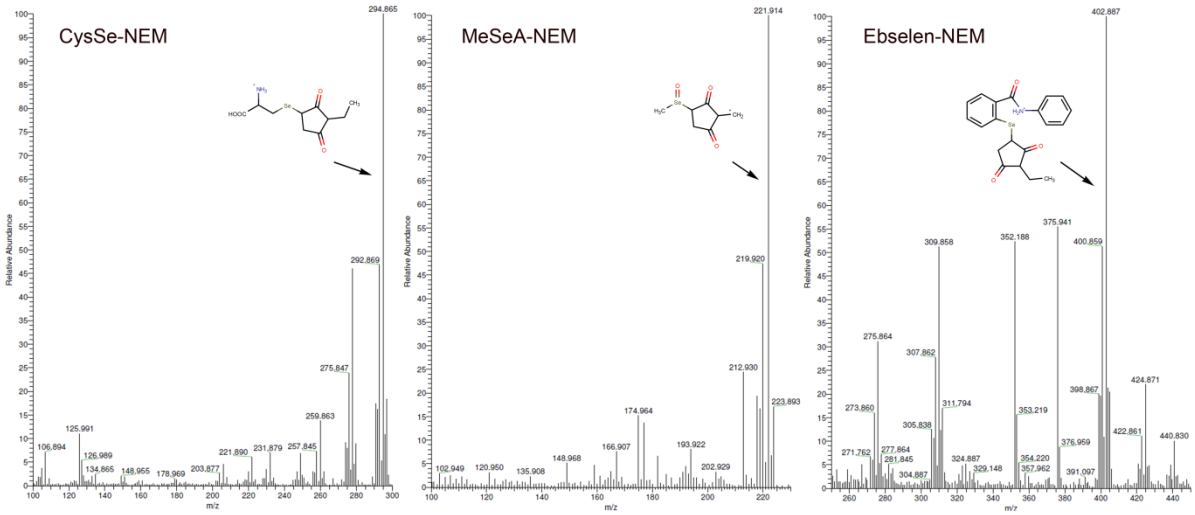
Supplementary Figure 2. Matrix effect on the ionization of selenocystine and methylseleninic acid. In the upper lane were the chromatograms of $CysSe_2$ and MeSeA injected through LC auto-sampler. Alternatively, to assess matrix effect on analyte ionization, $CysSe_2$ and MeSeA were continuously infused through MS syringe. In the same time, 2 µL of deproteinized extracts from plasma (middle lane) or 5% BSA (lower lane) were injected through LC auto-sampler. The chromatograms were acquired.



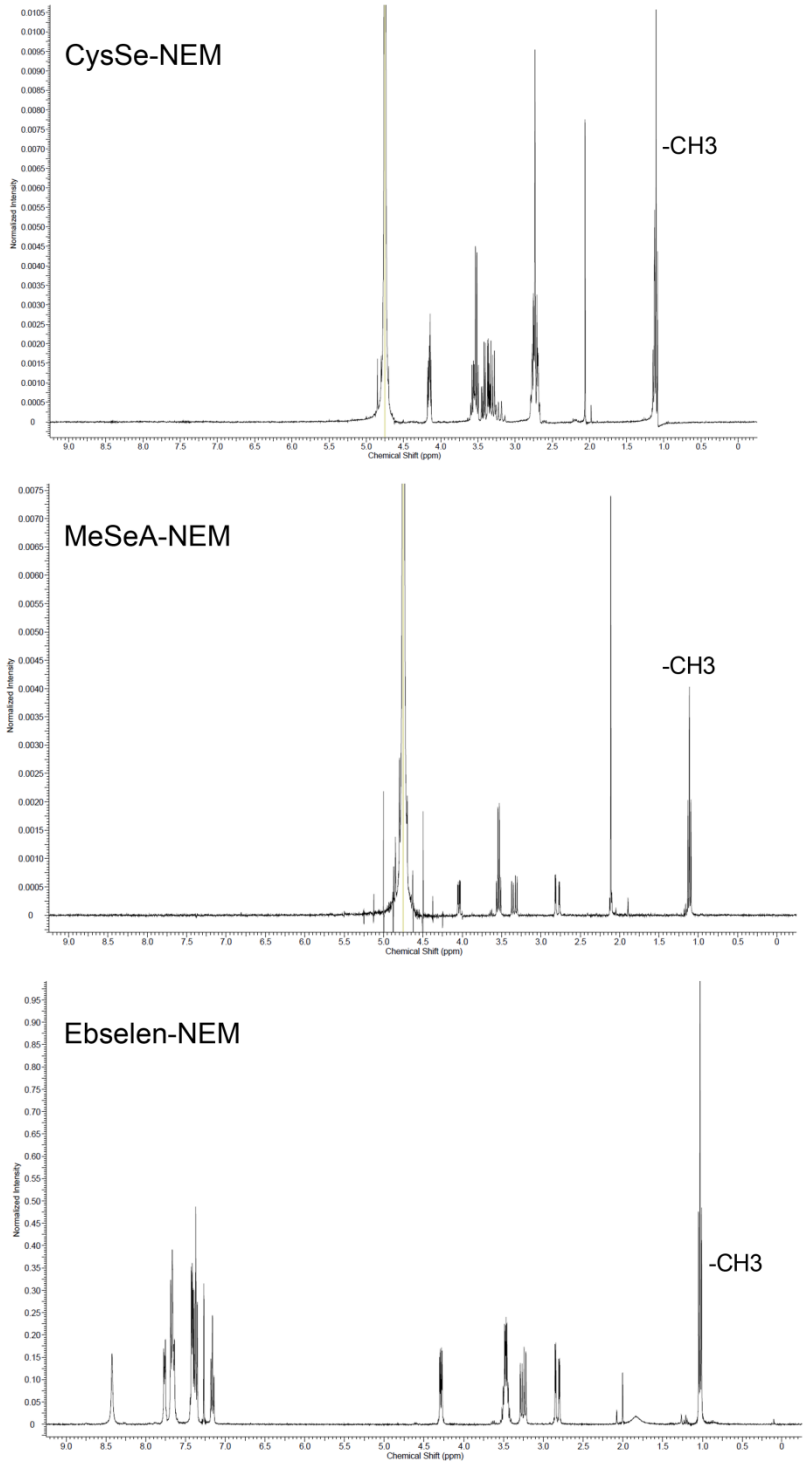
Supplementary Figure 3. X-ray absorption near edge spectra. Black, red and green lines indicate SeC, SeC-HSA and SeC-HP, respectively.



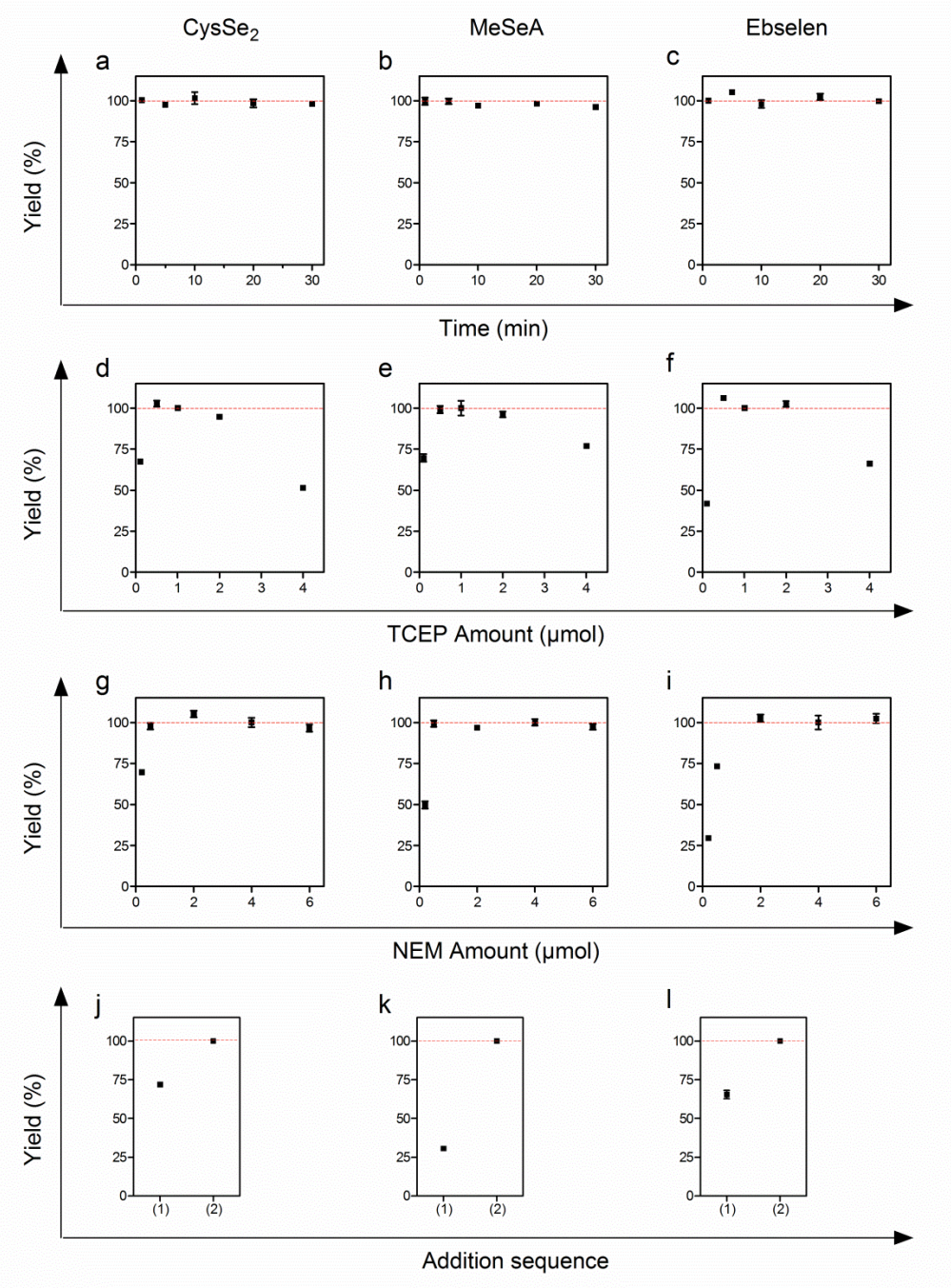
Supplementary Figure 4. Electrospray ionization-mass spectrometry spectrum of selenol derivative. SeC-NEM was dissolved in ACN at appropriate concentration and directly infused through MS syringe. The spectrum was acquired using full-scan Q1 mode (Peak Width of 0.7).



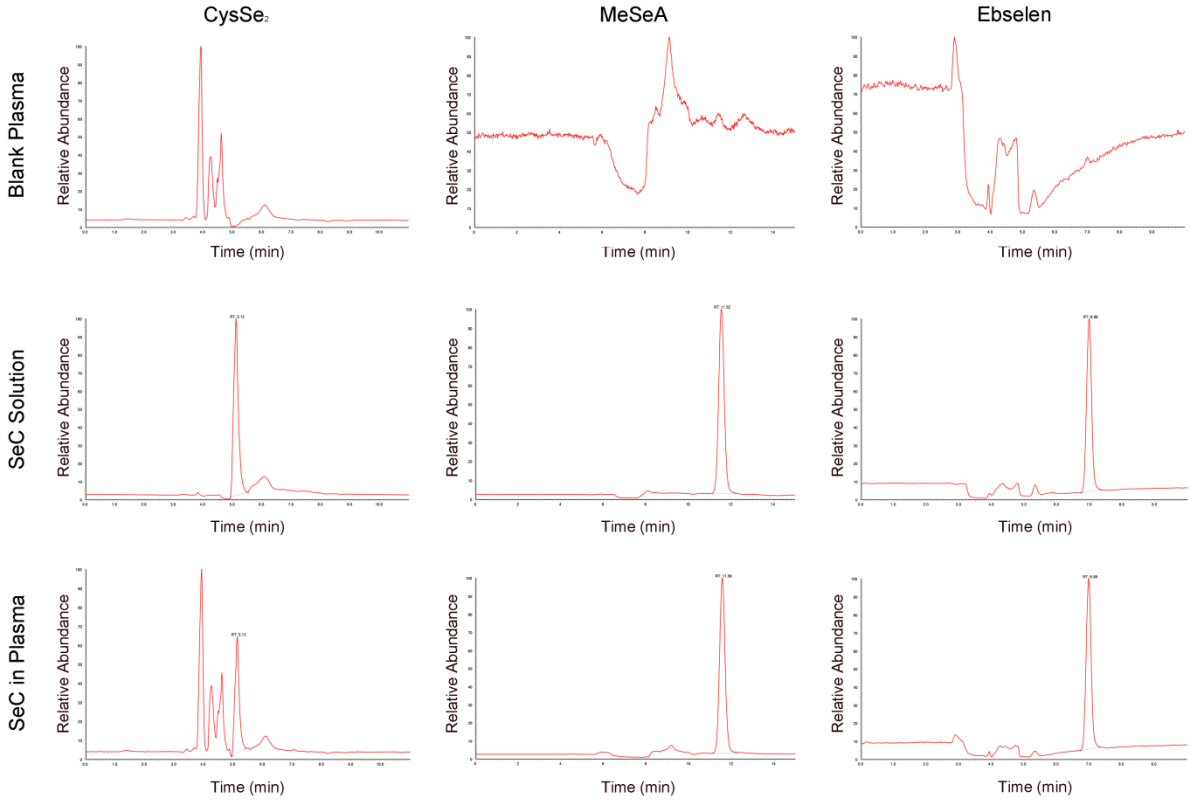
Supplementary Figure 5. ^{1}H-NMR spectrum of selenol derivative. CysSe-NEM and MeSeA-NEM were dissolved in D_2O, while Ebselen-NEM was dissolved in $CDCl_3$ for ^{1}H-NMR analysis (25 °C, 400 MHz, Bruker DRX-400, Germany).



Supplementary Figure 6. Optimization of RECID. (a-c) Yield of the derivative after different reaction times. TCEP and NEM amount were 1 µmol and 4 µmol, respectively. (d-f) Yield of the derivative at different TCEP concentrations. NEM concentration was 4 µmol and reaction time was 10 min. (g-i) Yield of the derivative at different NEM concentrations. TCEP concentration was 1 µmol, and the reaction time was 10 min. (j-l) Yield of the derivative upon changing the sequence in adding TCEP (1 µmol) and NEM (4 µmol): TCEP before NEM (1) or NEM before TCEP (2). The reaction time was 10 min. In experiments related to panel a-i, NEM was added before TCEP. In all experiments, derivative yield was shown as the percentage of peak area at indicated setting to that at optimal condition. Results are shown as the mean ± SD of three technical replicates.



Supplementary Figure 7. LC-MS chromatograms of SeC using RECID.



Supplementary Figure 8. Analysis of selenocompound in mouse and human plasma. SeC was spiked into blank human or mouse plasma (final concentration 10 µg/ml) and analyzed using RECID. Results are shown as the mean ± SD of three technical replicates.