Electronic supplementary information

Tracing cytotoxic effects of small organic Se species in human liver cells back to total cellular Se and Se metabolites

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Supplementary table 1: ICP-QQQ-MS parameters.

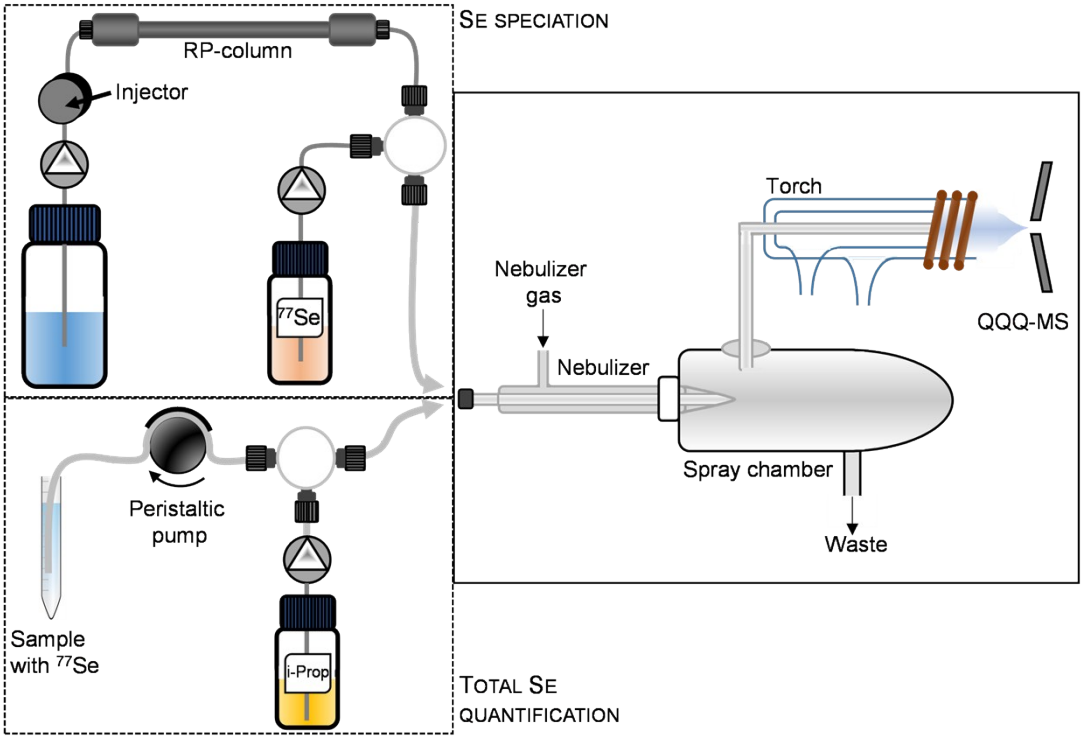
RF power	1550 W
Reflected power	< 5 W
Plasma gas flow	15 L/min
Carrier gas flow	1 L/min
Auxiliary gas flow	0.9 L/min
Collision and reaction gas mixture in the CRC	Oxygen (99.999%) 0.4 mL/min Hydrogen (99.999%) 1 mL/min
m/z (Q1 → Q2)	80 ⟶ 96 used for quantification 80 ⟶ 80 interference monitoring 78 ⟶ 94 interference monitoring 78 ⟶ 78 interference monitoring 77 ⟶ 93 spike 77 ⟶ 77 spike
Integration time	0.1 s
Number of sweeps	100
Sample flow	0.33 mL/min
Isopropanol flow	0.01 mL/min

Supplementary table 2: Quantitative investigation of the degradation of unknown species I and II applying HPLC-ICP-QQQ-MS analysis every 120 min with a 50 µM MeSeCys HepG2 cell lysate (4 d), which was stored at room temperature between the runs. As the volatile species DMSe shows enhanced response,[31] it was not included. (See also figure 3)

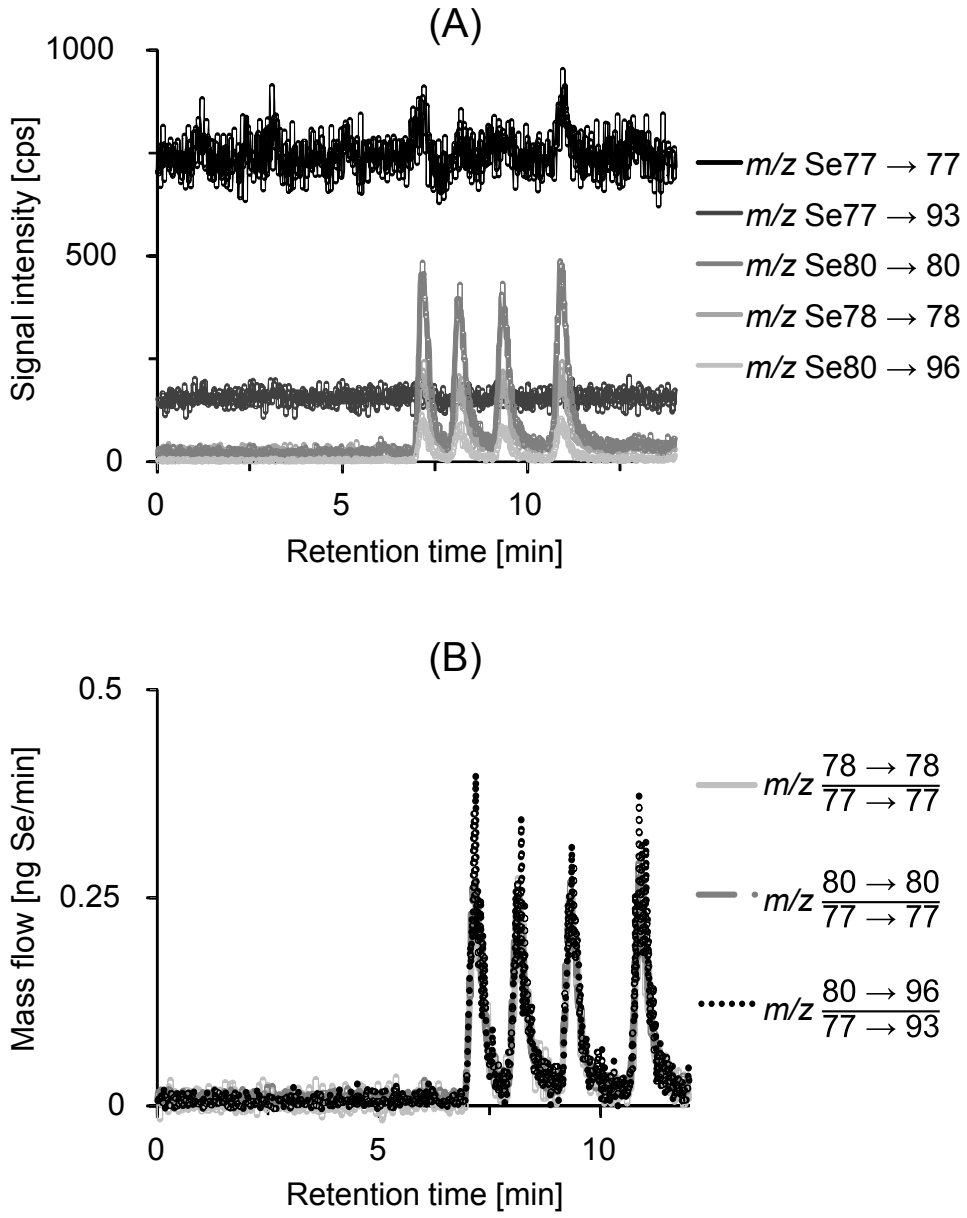
	Concentration in the measured solution [µg Se/L]			
Run #	MeSeCys	Unknown species I	Unknown species II	Sum [µg Se/L]
1	12.9	2.7	21.4	37.0
2	27.2	1.4	7.1	35.7
3	34.0	0.7		34.7
4	34.5			34.5

Supplementary table 3: Percentual comparison of total cellular Se concentrations to the amount of speciated Se compounds.

Incubation [µM]		time	Total cellular Se [µM]	Sum of small species [% of total cellular Se]	Amount of small Se species [% of total cellular Se]			Gap of sum of small species to total Se [% of total cellular Se]
MeSeCys	25	48 h	9	29	13 % MeSeCys	0,0 % unknown species I	15 % unknown species II	71
		4 d	12	40	22 % MeSeCys	0,0 % unknown species I	18 % unknown species II	60
	50	48 h	14	36	18 % MeSeCys	0,0 % unknown species I	18 % unknown species II	64
		4 d	20	42	20 % MeSeCys	2.5 % unknown species I	19 % unknown species II	58
	100	48 h	24	32	16 % MeSeCys	2.0 % unknown species I	14 % unknown species II	68
		4 d	65	62	27 % MeSeCys	6.0 % unknown species I	29 % unknown species II	38
SeMet	150	48 h	963	9,2	5.8 % SeOMet?	2.6 % SeMet	0.7 % unknown species III	91
		4 d	1664	9,7	6.6 % SeOMet?	2.6 % SeMet	0.5 % unknown species III	90
	200	48 h	1066	13	8.2 % SeOMet?	3.8 % SeMet	0.9 % unknown species III	87
		4 d	1664	14	8.2 % SeOMet?	4.7 % SeMet	0.8 % unknown species III	86
TMSe	500	4 d	99	94	94 % TMSe			6,2
SeSugar **1**	500	4 d	91	89	89 % SeSugar **1**			11



Supplementary figure 1: Instrumental set-up for Se speciation and total Se quantification using ID(-HPLC-)ICP-QQQ-MS.



Supplementary figure 2: Standard reference chromatogram of 5 µg/L MeSeCys (7.3 min), TMSe (8.4 min), SeMet (9.6 min) and SeSugar **1** (11.1 min) (A) and the corresponding mass flow chromatogram (B). Chromatographic separation was performed on a YMC-TriartPFP column (3 µm, 3x250 mm) at 30 °C with 250 µL/min 20 mM ammonium formate (pH 3, 3% methanol) as mobile phase.