Supplementary Information (Part 1) for:

Preparation and Certification of Natural and ^{82}Se-Labelled Selenomethionine Reference Materials

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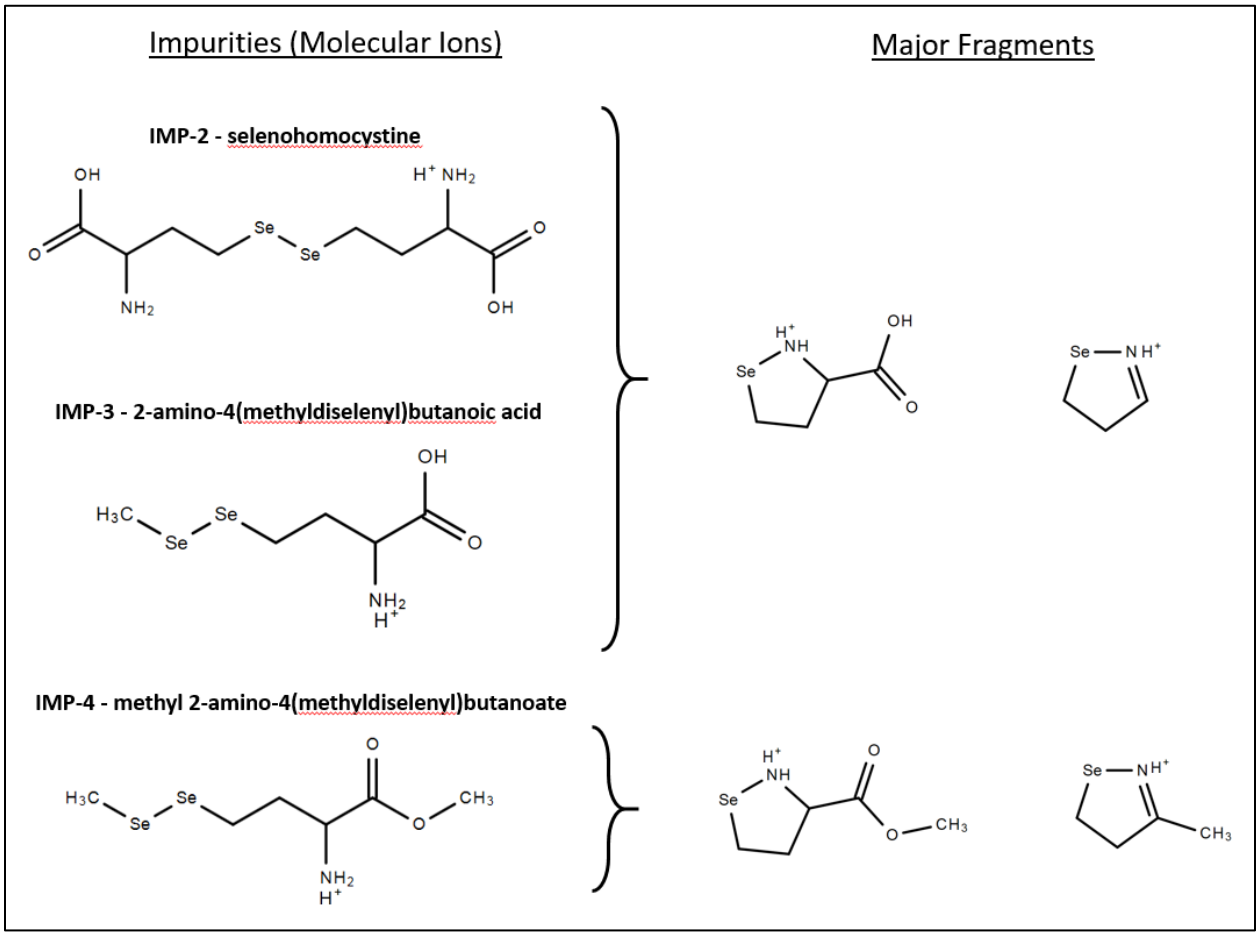

Figure S1: Major Se-containing impurities in SENS-1, showing each molecule's two main fragments.

Additional Information Relating to 1H NMR of SeMet in SENS-1

Initially, the purity of SeMet was measured using a combination of two resonances. Peak integrals were determined for the 1H corresponding to CH (4.00 – 3.45 ppm) and 7H corresponding to the combined H_3C-Se-CH_2-CH_2- signals (2.26 – 1.53 ppm) for SeMet and the 4H (7.90 – 7.20 ppm) signal for the internal standard; see Figure 5 in the main text.

Since the ^{1}H NMR signals for the impurities in SENS-1 could not be spectrally resolved from the SeMet-derived peaks, their contribution needed to be subtracted to ensure that the purity of SeMet was not overestimated. Standards of these species were not available, therefore the proposed chemical shifts noted in Figure 4 were predicted based on simulation and values obtained in the literature.[34] This information, along with the concentrations of these impurities (determined by HPLC-ICP-QQQ-MS as described in the section above) allowed their contribution to be subtracted from the SeMet peak areas.

Six samples of SENS-1 were measured and triplicate measurement per sample were performed. For each sample of the two SeMet signals, a mass fraction and associated uncertainty was calculated for each individual sample. The arithmetic average of the results for 1H at 4.00 – 3.45 ppm and 7H at 3.00 – 1.40 ppm was calculated and yield purities of 0.9785 and 0.9783 g/g respectively (Table 1). These two set results were then combined into a single purity result of 0.9784 g/g.

Table S1: ^{1}H-qNMR results of six independent samples of SeMet on Bruker 400 MHz, using KHP as internal standard (3 replicate measurements per sample)

Sample	1H at 4.00 – 3.45 ppm		7H at 3.0 – 1.40 ppm	
	Mean(g/g)	U_{1H} (g/g)	Mean(g/g)	U_{7H} (g/g)
1	0.9629	0.0036	0.9737	0.0014
2	0.9629	0.0036	0.9735	0.0013
3	0.9628	0.0036	0.9737	0.0015
4	0.9631	0.0036	0.9736	0.0014
5	0.9629	0.0036	0.9737	0.0014
6	0.9629	0.0036	0.9734	0.0014
Average	0.9629	0.0036	0.9736	0.0014

For the final result, the combined uncertainty was determined by the following equation:

$$U_c = \sqrt{\sum \frac{{u_i}^2}{N}}$$

where u_c is the combined uncertainty, u_i is the uncertainty of the individual result, and N is the number of results.

Uncertainty values were calculated to account for various components including signal recovery ($7T_1$), peak integration incompleteness, peak integration analyst, weighing of the sample, molar mass of SeMet, weighing of the standard, molar mass of standard, purity of standard KHP (Table S1).

The final purity value, based on qNMR was calculated to be 0.9684 g/g, with an expanded uncertainty (k=2) of 0.0050 g/g. It is important to note that the purity value calculated by qNMR could not account for the contribution of the minor impurities observed during HPLC-ICP-QQQ-MS analysis as the identities of these species are unknown. Additionally, when looking closely at the NMR spectrum it becomes clear that the significant amount of overlap from the impurities in the 3.0 – 1.4 ppm region contributes a large degree of uncertainty to the overall calculation. Based on this, it was determined that the best course of action was to use only 4.00 – 3.45 ppm signal, correcting this value based on the contributions of the four main impurities as well as the modelled data for "Impurity-X", the sum of the unidentified species.

Table S2: ^{1}H-qNMR-uncertainty components considered for SENS-1

Number	**Uncertainty component**	***u* (g/g)**
1	Repeatability of measurement	0.00131
2	Signal recovery ($7T_1$)	0.0010
3	Peak integration (incompleteness)	0.0010
4	Peak integration (analyst, methionine)	0.0005[a]
5	Method uncertainty due to different signals	0.0050
6	Weighing of the analyte (SENS-1)	0.0002
7	Weighing of the calibrant (KHP)	0.0003
8	Molar mass of calibrant (KHP)	0.0000
9	Purity of standard (KHP)	0.0000
10	Molar mass of analyte (SENS-1)	0.0000
11	13C satellites	0.0000
	Overall combined uncertainty	**0.0028**

The Model of SENS-1 Data Combination and Purity Determination

// HPLC data for Met mass fraction in SENS-1 (g/g)

i1 ~ normal(wMet, u(wMet))

// HPLC-ICPMS impurity data for peak areas relative to the SeMet

i2 ~ normal(R2, 0.2*R2)

i3 ~ normal(R3, 0.2*R3)

i4 ~ normal(R4, 0.2*R4)

iX ~ normal(RX, 0.2*RX)

// 1H-qNMR data for SeMet and interfering impurities (g/g SENS-1)

// hi is the effect of homogeneity in the results from seven CRM units

wNMR,i ~ N(wNMR + hi, uNMR) where i = 1…7

// ICPMS data for total Se in SENS-1 (g/g)

// hi is the effect of homogeneity in the results from three CRM units

wICPMS,i ~ N(wtotal + 0.4*hi+7, uICPMS) where i = 1…3

These data are then transformed to the equivalent mass fraction of SeMet:

r1 = wtotal*(MSeMet/MSe)/(1 + R2 + R3 + R4 + RX)

r2 = wNMR – (MSeMet)*(1*wMet/MMet + 2*wImp2/M2 + 1*wImp3/M3 + 4*wImp4/M4 + NX*wImpX/MX)

The mass fractions of impurities 2, 3, 4, and X in SENS-1 are calculated as follows:

wImp2 = wtotal*(M2/2)*(R2/(1 + R2 + R3 + R4 + RX))/MSe;

wImp3 = wtotal*(M3/2)*(R3/(1 + R2 + R3 + R4 + RX))/MSe;

wImp4 = wtotal*(M4/2)*(R4/(1 + R2 + R3 + R4 + RX))/MSe;

wImpX = wtotal*(MX/Z)*(RX/(1 + R2 + R3 + R4 + RX))/MSe;

The corrected mass fractions of SeMet, r1 and r2, are then further modeled as a random draw from normal distribution whose mean is the final mass fraction of SeMet in SENS-1:

r ~ N(wSeMet_grand, uSeMet_grand)

Model Output for SENS-1 Data from ICP-MS and qNMR Methods

Table S3: Statistical information associated with the determination of the selenomethionine concentration in SENS-1 by two analysis methods, ICP-MS and qNMR.

	ICP-MS	**qNMR**
Mean ($g\ g^{-1}$)	0.96236	0.95930
Standard Deviation ($g\ g^{-1}$)	0.00218	0.00273
2.5% CI ($g\ g^{-1}$)	0.95801	0.95361
97.5% CI ($g\ g^{-1}$)	0.96670	0.96427
n_{eff}	3601	2879
R_{hat}	1.00012	1.00102