Supporting Information to

Accurate high throughput quantification of selenium in biological samples- the potential of combining isotope dilution ICP-tandem mass spectrometry with flow injection

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Table S1. Precision for each Se isotope ratio is determined from a CRM blend by four consecutive measurements in H_2 and O_2 -ICP-MS/MS mode. CRM blends were prepared and digested as described in the experimental section.

Precision of isotope ratios from CRM blend					
Gas	H_2		O_2		
CRM blend	$^{78}Se/^{77}Se$	$^{80}Se/^{77}Se$	$^{78}Se/^{77}Se$	$^{80}Se/^{77}Se$	$^{82}Se/^{77}Se$
Average (n=4)	0.26	0.59	0.24	0.54	0.10
STDEV	0.003	0.006	0.003	0.002	0.002
RSD [%]	1.13	1.11	1.11	0.35	1.96
Mass Bias [%]	3.87	12.6	2.64	13.9	17.6

Figure S1. (1) Isotope dilution equation employed for calculating concentration of selenium in direct infusion. The concentration of the spike (C_{spk}) was calculated by reverse IDMS, whereas (W_{spk}) and (W_{std}) respectively refer to the weights of spike and standard. *R* refers to the ratio reference-to-enriched isotope into the blend. (h_y) refers to the abundance of the enriched isotope while (h_x)is the IUPAC correspondent abundance. **(2)** Equation employed for calculating the analyte mass flow (*Mx (t)*). **(3)** The mass flow of the spike (*My (t)*) was assessed by multiplying C_{spk} with the make-up flow rate (f_{sp}) **(4)** Selenium concentration was calculated by dividing absolute amounts (M_{xj}) as integrated between *t1* and *t2*, through the injection volume (*IV*). **(5)** (*K*) Mass bias correction was calculated in external calibration: a 100 µg L^{-1} Se-standard was measured 7 times consecutively and the true ratio (R_{true}) was divided by the observed ratio (R_{obs}).

(1)

$$C = \frac{C_{spk} * W_{spk}}{W_{std}} * \frac{R_y - R_b * K}{K * R_b - R_x} * \frac{h_{iy}}{h_{ix}}$$

(2)

$$M_x(t) = M_y(t) * \frac{R_y - R_b * K}{K * R_b - R_x} * \frac{h_{iy}}{h_{ix}}$$

(3)

$$M_y(t) = f_{sp} * C_{spk}$$

(4)

$$C = \frac{\int_{t1}^{t2} M_{xj}}{IV}$$

(5)

$$K = \frac{R_{true}}{R_{obs}}$$

Table S2. Table reporting value/unit and respective standard uncertainties for each factor included in the TCU. Quantification parameters as well as variables are reported in model equation (1).

H_2 mode

		^{78}Se		^{80}Se		^{82}Se	
Quantification parameter	Variables	Value/unit	Standard uncertainty	Value/unit	Standard uncertainty	Value/unit	Standard uncertainty
Weight of spike	W_{spk}	198.2 mg	0.002	198.2 mg	0.002	198.2 mg	0.002
Weight of CRM/serum	W_{crm}	182.65 mg	0.002	182.65 mg	0.002	182.65 mg	0.002
Concentration of isotopically enriched 77Se "spike"	C_{spk}	0.67 µmol L^{-1} (n=5)	0.026	0.68 µmol L^{-1} (n=5)	0.024	0.71 µmol L^{-1} (n=5)	0.041
Isotopic abundance of the reference isotope in the spike	A_{spk}	0.3 % (certificate)	0.020	0.2 % (certificate)	0.020	0.2 % (certificate)	0.020
Isotopic abundance of the enriched isotope in the spike	B_{spk}	99.2 % (certificate)	0.020	99.2 % (certificate)	0.020	99.2 % (certificate)	0.020
Isorope ratio in the test blend	R_b	0.219 (n=5)	0.003	0.500 (n=5)	0.006	0.097 (n=5)	0.002
Mass bias	K	1.08 (n=7)	0.005	0.986 (n=7)	0.005	0.935 (n=7)	0.006

O_2 mode

		^{78}Se		^{80}Se		^{82}Se	
Quantification parameter	Variables	Value/unit	Standard uncertainty	Value/unit	Standard uncertainty	Value/unit	Standard uncertainty
Weight of spike	W_{spk}	200.04 mg	0.002	200.04 mg	0.002	200.04 mg	0.002
Weight of CRM/serum	W_{crm}	191.93 mg	0.002	191.93 mg	0.002	191.93 mg	0.002
Concentration of isotopically enriched 77Se "spike"	C_{spk}	0.77 µmol L^{-1} (n=5)	0.039	0.79 µmol L^{-1} (n=5)	0.018	0.85 µmol L^{-1} (n=5)	0.074
Isotopic abundance of the reference isotope in the spike	A_{spk}	0.3 % (certificate)	0.020	0.2 % (certificate)	0.020	0.2 % (certificate)	0.020
Isotopic abundance of the enriched isotope in the spike	B_{spk}	99.2 % (certificate)	0.020	99.2 % (certificate)	0.020	99.2 % (certificate)	0.020
Isorope ratio in the test blend	R_b	0.204 (n=5)	0.003	0.491 (n=5)	0.002	0.085 (n=5)	0.002
Mass bias	K	1.05 (n=7)	0.004	0.913 (n=7)	0.003	0.85 (n=7)	0.003

Figure S2. Uncertainty budget "pies": each pie illustrates the contribution brought to respectively ^{78}Se, ^{80}Se, ^{82}Se in either gas mode as given by I- Weight of spike. II- Weight of CRM. III- Concentration of selenium in spike. IV- Isotopic abundance of reference isotope in spike. V- Isotopic abundance of enriched isotope in spike. VI- Blend isotope ratio. VII- Mass bias.