Supplement S1

Species unspecific post column isotope dilution analysis (IDA)

The equation used for calculation of the mass flow chromatogram are from the tutorial of Rodriguez-Gonzales et al.[1]

The quantitation of Se-PenM and Pen included a conversion of the chromatogram obtained from the ICP-MS/MS analysis into a mass flow chromatogram. The set-up is exemplified for Se analysis and was performed in the same way for S analysis. The instrumental parameters described in the experimental section were used.

The purpose of post-column IDA is to convert the chromatogram (signal vs. time) to a mass flow chromatogram (mass vs. time). After separation of the Se species (^{80}Se) in the chromatographic column, the spike solution containing the enriched Se isotope (^{77}Se) was mixed with the eluent via a T-piece prior to entering the ICP-MS. After reaction with the cell gas O_2, the two Se isotopes were monitored at *m/z* 96 ($^{80}Se^{16}O$) and *m/z* 93 ($^{77}Se^{16}O$), respectively.

Isotope measurements were corrected for blank, instrument dead time and mass bias. The Agilent 8800 corrects for dead time automatically.

Mass bias correction

The experimental isotope ratio was corrected using the mass bias factor as shown in Equation 1:

$$R_{corrected} = R_{measured} \cdot exp(F \cdot \Delta m) \qquad \textbf{(Equation 1)}$$

- $R_{corrected}$ is the corrected and $R_{measured}$ is the measured isotope ratio (a/b), respectively
- Δm is the mass difference between the isotopes
- *F* is the mass bias factor per mass unit

The mass bias factor per mass unit, *F,* was calculated, as described below, and applied for correction of the experimental isotope ratio of ($^{80}Se^{16}O$)/($^{77}Se^{16}O$) for each measurement point on the entire chromatogram. The average signal intensities of a standard Se-solution corrected for blank signals was used to calculate the experimental isotope ratio and the theoretical isotope ratio was calculated from the natural abundances [2].

An example from a random analysis is given below:

$$R_{experimental} = \frac{Intensity - Se^{16}O - blank\ ^{80}Se^{16}O}{Intensity\ ^{77}Se^{16}O - blank\ ^{77}Se^{16}O} = \frac{526756 - 630}{78211 - 434} = 6.76$$

$$R_{theoretical} = \frac{49.61}{7.63} = 6.50$$

The mass bias factor for the experiment was calculated by rearranging Equation 1:

$$F = \frac{ln(\frac{6.50}{6.76})}{-3} = 0.013$$

The mass bias factor corresponds to -1.3 % per unit mass.

The mass bias factor was calculated approximately every third hour and varied between 0.5 and 2.6 % for Se and 1.3-3.8 % for S, respectively, during the experimental period.

Mass flow chromatograms

Subsequent to blank and mass bias correction of the isotope ratio in the mixture, the isotope dilution equation, Equation 2, was applied to the isotope ratios of $^{80}Se^{16}O/^{77}Se^{16}O$ from the entire chromatogram to obtain the mass flow data:

$$MF_x = c_{Sp} \cdot d_{Sp} \cdot f_{Sp} \cdot \frac{AW_x}{AW_{Sp}} \cdot \frac{A_{Sp}^b}{A_x^a} \cdot \left(\frac{R_m - R_{Sp}}{1 - R_m \cdot R_x}\right) \qquad \text{(Equation 2)}$$

- MF_x is the mass flow of the sample
- c_{Sp} is the concentration of the enriched isotope standard used as spike-solution
- d_{Sp} and f_{Sp} are the density and the flow rate of the spike-solution, respectively
- AW_{SP} and AW_x are the atomic masses of the element in the spike-solution and the sample, respectively
- A_{Sp}^b and A_x^a are the isotope abundances of b in the spike-solution and a in the sample, respectively
- R_m is the isotope ratio (a/b) in the mixture after mass bias correction
- R_{Sp} is the theoretical isotope ratio in the spike-solution (a/b) and is defined as $R_{Sp} = A_{Sp}^a / A_{Sp}^b$
- R_x is the theoretical isotope ratio in the sample (b/a) and is defined as $R_x = A_x^b / A_x^a$.

c_{sp}, ,the concentration of the ^{77}Se-enriched spike-solution was determined by reverse isotope dilution. The spike-solution was diluted to a concentration of 141.2 µg L^{-1}
d_{Sp}, the density of the spike solution was 1.00 g mL^{-1} and f_{Sp} the flow rate of the spike-solution was 10 µL min^{-1}
AW_{SP} and AW_x, the atomic masses of the element in the spike-solution and the sample, were 76.92 g/mol and 79.92 g/mol, respectively
A_{Sp}^b and A_x^a , the isotope abundances of b in the spike-solution and a in the sample, were 0.992 and 0.496, respectively
R_m, the isotope ratio (a/b) in the mixture after mass bias correction
R_{Sp}, the theoretical isotope ratio in the spike-solution (a/b) defined as $R_{Sp} = A_{Sp}^a / A_{Sp}^b$ was 0.002/0.992
R_x, the theoretical isotope ratio in the sample (b/a) defined as $R_x = A_x^b / A_x^a$ was 7.63/49.61

The mass flow data was plotted against time to obtain the mass flow chromatogram.
Integration of the whole mass flow chromatogram results in the total amount of the element in the sample. The amounts, corresponding to the various peaks, were converted via the injection volume.

1. P. Rodríguez-González, J. M. Marchante-Gayón, J. I. García Alonso and A. Sanz-Medel, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2005, **60**, 151-207.
2. M. Berglund and M. E. Wieser, *Pure and Applied Chemistry*, 2011, **83**, 397-410.