Electronic Supplementary Information (ESI)

Preparation and characterisation of ^{57}Fe enriched haemoglobin spike material for species-specific isotope dilution mass spectrometry

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Stability of the HGB-spike

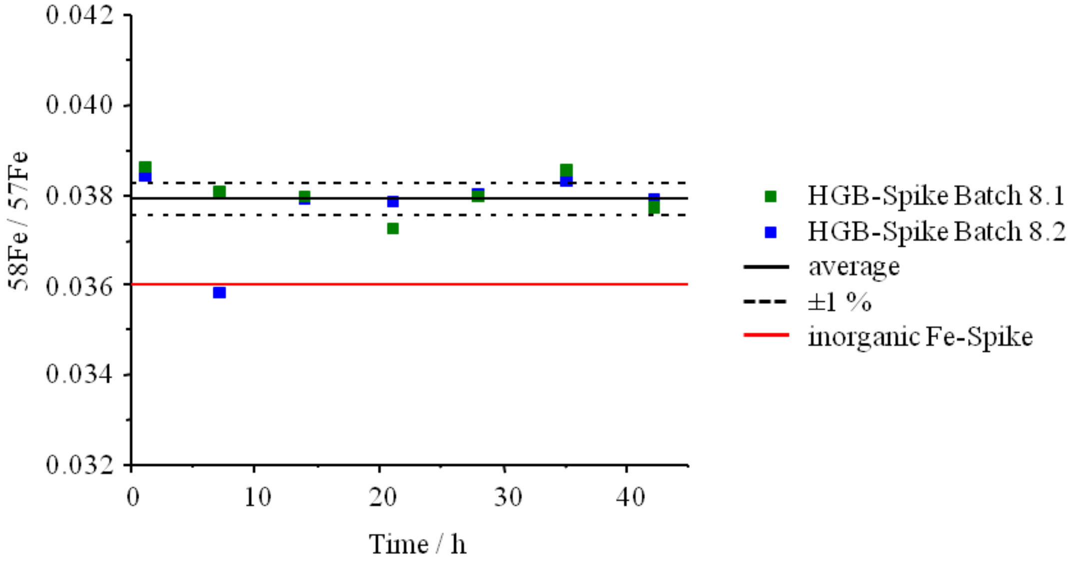

Figure ESI-1: Stability investigation of HGB spike of two different aliquots from the same batch (batch 8) using MonoQ®-ICP-MS. Batch 8.1 was dissolved in 12.5 mol Tris-buffer pH 7.8 and stored at -20 °C for 5 months, batch 8.2 was dissolved prior to the measurement. Isotopic ratio of $^{58}Fe/^{57}Fe$ the HGB-spike of batch 8.1 and batch 8.2 over a time period of 42 h is illustrated.

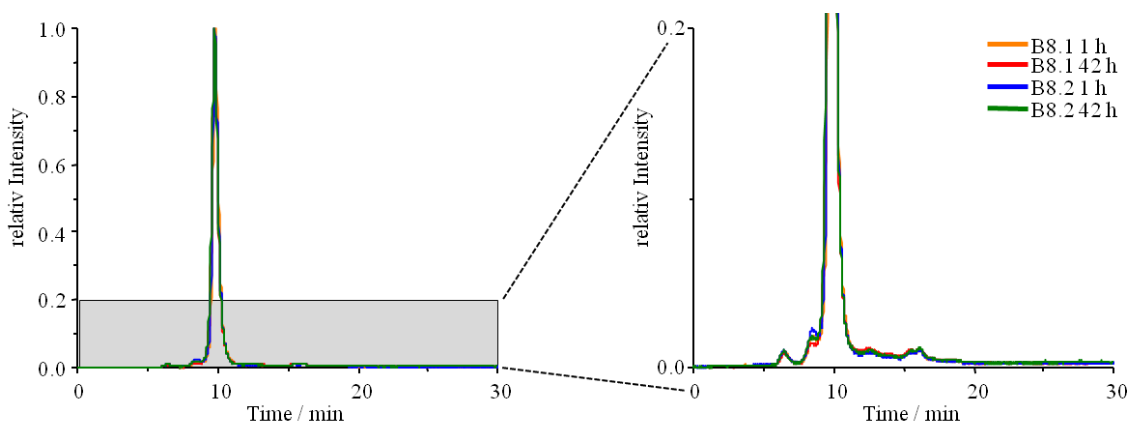

Figure ESI-2: MonoQ®-ICP-MS chromatograms of the HGB spike derivatised with KCN. In the close up the additional Fe peaks are shown which are stabile during the analysis time and no further peaks appear.

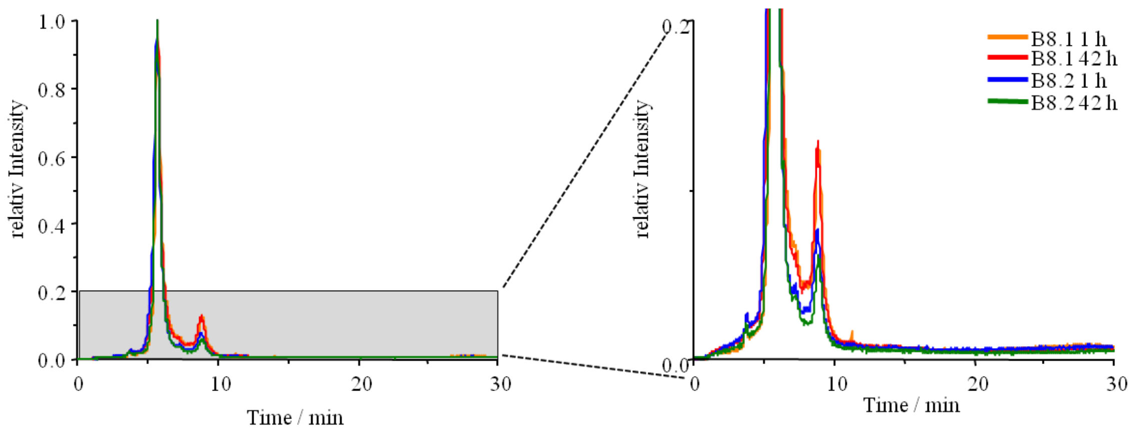

Figure ESI-3: MonoQ®-ICP-MS chromatograms of the HGB spike. In the close up the additional Fe peaks are shown which are stabile during the analysis time and no further peaks appear.

HGB-spike applied in species-specific IDMS

In the following the equations for the evaluation of the double [1] (equation 1) and triple [2, 3] (equation2) IDMS approaches are shown.

Equation 1: Double IDMS:

$$w_\mathrm{x} = w_\mathrm{z} \cdot \frac{m_\mathrm{yx}}{m_\mathrm{x}} \cdot \frac{m_\mathrm{z}}{m_\mathrm{yz}} \cdot \frac{R_\mathrm{y} - R_\mathrm{bx}}{R_\mathrm{bx} - R_\mathrm{x}} \cdot \frac{R_\mathrm{bz} - R_\mathrm{z}}{R_\mathrm{y} - R_\mathrm{bz}}$$

Equation 2: Triple IDMS:

$$w_\mathrm{x} = w_\mathrm{z} \cdot \frac{m_\mathrm{yx}}{m_\mathrm{x}} \cdot \frac{1}{R_\mathrm{x} - R_\mathrm{bx}} \left[\frac{m_\mathrm{z1}}{m_\mathrm{yz1}} \cdot \frac{R_\mathrm{bz2} - R_\mathrm{bx}}{R_\mathrm{bz2} - R_\mathrm{bz1}} \cdot \left(R_\mathrm{x} - R_\mathrm{bz1}\right) + \frac{m_\mathrm{z2}}{m_\mathrm{yz2}} \cdot \frac{R_\mathrm{bx} - R_\mathrm{bz1}}{R_\mathrm{bz2} - R_\mathrm{bz1}} \cdot \left(R_\mathrm{x} - R_\mathrm{bz2}\right) \right]$$

Symbol explanation for equation 1 & 2:

Symbol (Unit)	Quantity
w_x, w_z (g/kg)	Mass fraction of Fe in sample x, spike y and the references z
m_x, m_z, (g)	Mass of the solutions of sample x and reference z in the blends
m_{yx}, m_{yz}, (g)	Mass of spike solution used to prepare the blends bx (sample x + spike y) and bz, bz1, bz2 (reference z + spike y)
R_x, R_y, R_z (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in sample x, spike y and reference z
R_{bx} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blend bx (sample x + spike y)
R_{bz1}, R_{bz2} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blends bz1 and bz2 (reference z + spike y) for triple IDMS
R_{bz} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blend bz (reference z + spike y) for double IDMS

Uncertainty budget of double IDMS of IRMM/IFCC-467by means of SEC-ICP-MS calculated with GUM workbench:

Result: w_x: **mass fraction of HGB in IFCC-467**

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
m_{yx}	0.02909 g	$14.5\cdot10^{-6}$ g	normal	4200	0.061 mg/g	0.5 %
m_x	0.02974 g	$14.9\cdot10^{-6}$ g	normal	-4100	-0.061 mg/g	0.5 %
m_z	0.02964 g	$14.8\cdot10^{-6}$ g	normal	4100	0.061 mg/g	0.5 %
m_{yz}	0.02819 g	$14.1\cdot10^{-6}$ g	normal	-4300	-0.061 mg/g	0.5 %
R_y	174.63 mol/mol	0.290 mol/mol	normal	$-380\cdot10^{-6}$	$-110\cdot10^{-6}$ mg/g	0.0 %
m_{IFCC}	0.05054 g	$25.3\cdot10^{-6}$ g	normal	-2400	-0.061 mg/g	0.5 %
m_{xv}	1.49226 g	$746\cdot10^{-6}$ g	normal	81	0.061 mg/g	0.5 %
x_{z57}	0.021191mol/mol	$32.5\cdot10^{-6}$ mol/mol	normal	11	$350\cdot10^{-6}$ mg/g	0.0 %
x_{z56}	0.91754 mol/mol	$180\cdot10^{-6}$ mol/mol	normal	-0.25	$-45\cdot10^{-6}$ mg/g	0.0 %
R_{bx}	1.05559 mol/mol	$5.76\cdot10^{-3}$ mol/mol	normal	-120	-0.68 mg/g	59.8 %
R_{bz}	1.15079 mol/mol	$2.96\cdot10^{-3}$ mol/mol	normal	110	0.32 mg/g	13.2 %
k_{mg}	1000.0 mg/g					
w_{pur}	0.4794 kg/kg	$1.68\cdot10^{-3}$ kg/kg	normal	250	0.43 mg/g	23.2 %
m_{HBA0}	0.01134 g	$5.67\cdot10^{-6}$ g	normal	11000	0.061 mg/g	0.5 %
m_{Lsg}	1.48415 g	$742\cdot10^{-6}$ g	normal	-82	-0.061 mg/g	0.5 %
w_x	121.555 mg/g	0.883 mg/g				

Uncertainty budget of triple IDMS of IRMM/IFCC-467 by means of SEC-ICP-MS calculated with GUM workbench:

Result: w_x: **mass fraction of HGB in IRMM/IFCC-467**

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
m_{yx}	0.02909 g	$14.5 \cdot 10^{-6}$ g	normal	4200	0.061 mg/g	0.4 %
m_x	0.02974 g	$14.9 \cdot 10^{-6}$ g	normal	-4100	-0.061 mg/g	0.4 %
m_{z1}	0.02964 g	$14.8 \cdot 10^{-6}$ g	normal	890	0.013 mg/g	0.0 %
m_{yz1}	0.02819 g	$14.1 \cdot 10^{-6}$ g	normal	-940	-0.013 mg/g	0.0 %
m_{z2}	0.03237 g	$16.2 \cdot 10^{-6}$ g	normal	2900	0.048 mg/g	0.2 %
m_{yz2}	0.02744 g	$13.7 \cdot 10^{-6}$ g	normal	-3500	-0.048 mg/g	0.2 %
x_{57}	0.02119 mol/mol	$50.0 \cdot 10^{-6}$ mol/mol	normal	-0.27	$-13 \cdot 10^{-6}$ mg/g	0.0 %
x_{56}	0.91754 mol/mol	$180 \cdot 10^{-6}$ mol/mol	normal	$6.1 \cdot 10^{-3}$	$1.1 \cdot 10^{-6}$ mg/g	0.0 %
k_{conv}	1000.0 mg/g					
w_{pur}	0.47940 g/g	$1.68 \cdot 10^{-3}$ g/g	normal	250	0.43 mg/g	19.2 %
m_{HBA0}	0.01134 g	$5.67 \cdot 10^{-6}$ g	normal	11000	0.061 mg/g	0.4 %
m_{Lsg}	1.48415 g	$742 \cdot 10^{-6}$ g	normal	-82	-0.061 mg/g	0.4 %
m_{IFCC}	0.05054 g	$25.3 \cdot 10^{-6}$ g	normal	-2400	-0.061 mg/g	0.4 %
m_{xv}	1.49226 g	$746 \cdot 10^{-6}$ g	normal	81	0.061 mg/g	0.4 %
R_{bx}	1.13914 mol/mol	$6.22 \cdot 10^{-3}$ mol/mol	normal	-110	-0.68 mg/g	49.6 %
R_{bz2}	1.11060 mol/mol	$5.78 \cdot 10^{-3}$ mol/mol	normal	88	0.51 mg/g	27.8 %
R_{bz1}	1.24188 mol/mol	$3.19 \cdot 10^{-3}$ mol/mol	normal	22	0.070 mg/g	0.5 %
w_x	121.576 mg /g	0.971 mg /g				

Symbol explanation for both uncertainty budgets:

Symbol (Unit)	Quantity
w_x, w_z (g/kg)	Mass fraction of Fe in sample x, spike y and the references z
m_x, m_z, (g)	Mass of the solutions of sample x and reference z in the blends
m_{yx}, m_{yz}, (g)	Mass of spike solution used to prepare the blends bx (sample x + spike y) and bz, bz1, bz2 (reference z + spike y)
R_x, R_y, R_z (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in sample x, spike y and reference z
R_{bx} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blend bx (sample x + spike y)
R_{bz1}, R_{bz2} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blends bz1 and bz2 (reference z + spike y) for triple IDMS
R_{bz} (mol/mol)	Isotope amount ratio of the analyte ($^{57}Fe/^{56}Fe$) in the blend bz (reference z + spike y) for double IDMS
x_{z57}, x_{z56} (mol/mol)	Amount-of substance fraction of the isotopes 56 and 57, resp., in natural Fe
w_{pur} (kg/kg)	Purity of the HGB reference material
m_{HBA0}, m_{Lsg}	Mass of the solution steps of the reference

References:

1. M. Sargent, C. Harrington and R. Harte, *Guidelines for achieving high accuracy in isotope dilution mass spectrometry (IDMS)*, Royal Sociaety of Chemistry, Cambridge, 2002.
2. C. Frank, O. Rienitz, C. Swart and D. Schiel, *Anal Bioanal Chem*, 2013, **405**, 1913-1919.
3. J. Vogl, *J Anal Atom Spectrom*, 2007, **22**, 475-492.