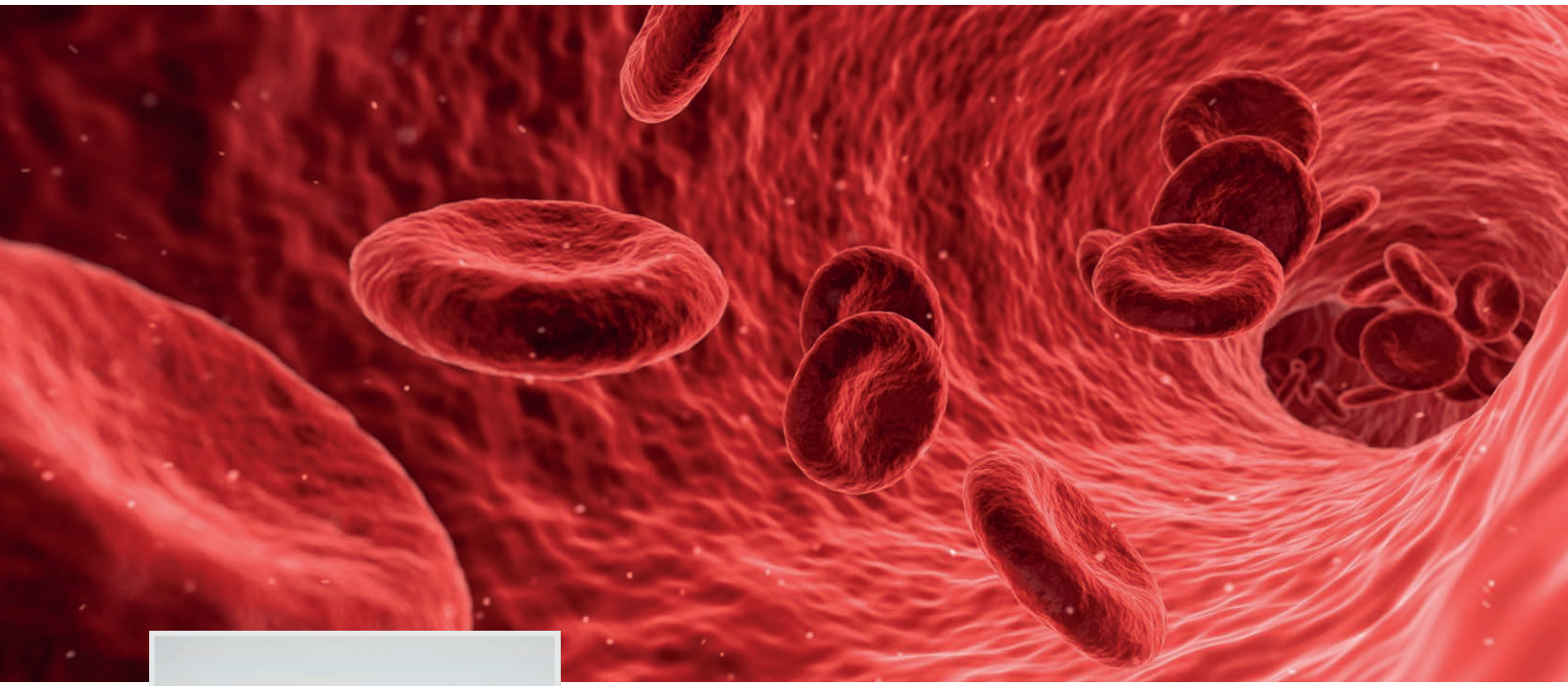

Challenge

Eliminating interferences for accurate and precise determination of Fe isotopes in whole blood

Solution

Robust cool plasma performance combined with integrated Collision Reaction Cell

Iron Isotope Ratios in Human Whole Blood by PlasmaQuant® MS

Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) is now a well-established technique for use in human studies because it can determine both the total elemental content and isotopic ratios with sufficient precision and accuracy in a single analytical run. Stable Fe isotope ratio determinations by ICP-MS in blood have attracted much interest in recent years, especially in human nutrition research where Fe stable isotopes are used to trace metabolic fate as a safer alternative to previously used radioisotopes. Because the precision of isotope analysis and mass spectrometric sensitivity are key issues that determine isotope dosage and the capacity to obtain meaningful isotopic enrichment after isotope administration, high precision and accurate isotopic measurements of at least three Fe isotopes are required.

Fe isotopic analysis by ICP-MS is a real analytical challenge because of the numerous and severe isobaric and polyatomic interferences on the four stable isotopes. In addition to Ar based molecular ions having the same nominal mass-to-charge ratio as the target isotopes (^{54}Fe, ^{56}Fe, ^{57}Fe, and ^{58}Fe) including $^{40}Ar^{14}N$, $^{38}Ar^{16}O$, $^{40}Ar^{16}O$, $^{38}Ar^{18}OH^+$, $^{40}Ar^{16}OH^+$, and $^{40}Ar^{18}O^+$, the presence of high concentrations of concomitant elements in blood samples, such as calcium, can affect the precision and accuracy of Fe isotopic ratios by the formation of $^{40}Ca^{16}O^+$, $^{40}Ca^{16}OH^+$, $^{40}Ca^{18}O^+$, and $^{42}Ca^{16}O^+$ in the ICP plasma. The presence of Cr and Ni in relatively low concentrations ($\mu g.L^{-1}$) in blood samples have also been found to significantly affect the accuracy of ratios involving $^{54}Fe^+$ and $^{58}Fe^+$ isotopes, unless additional corrections are applied or matrix separation performed before analysis. Several approaches have been applied for the determination of Fe isotopes in organic matrices.

analytikjena
An Endress+Hauser Company

Cool plasma conditions and membrane desolvation were reported by Vanhaecke et al. to offer insufficient reduction in the intensity of the interfering ions. However, the precision obtained was found sufficient only for single tracer experiments or for isotope dilution purposes. Instrumental approaches to resolve most of the spectral interferences on Fe isotopes such as the use of ion-molecule chemistry in collision/reaction cell constitute the most effective approaches.

This study combines the techniques of cool plasma and collision/reaction cell to completely eliminate polyatomic interferences and achieve the necessary precision and accuracy in the determination of Fe isotopes to distinguish between male and female whole blood samples.

Instrumentation

All samples were digested by Topwave® microwave digestion system using the settings displayed in table 1.

Table 1: Method parameters microwave digestion

Parameter	Specification
Sample Volume	1 mL
HNO_3	6 mL
H_2O_2	2 mL
Vessel	PL 100
Heating Stage 1 / Time	175°C / 5 min
Heating Stage 2 / Time	200°C / 30 min
Heating Stage 3 / Time	50°C / 20 min
Final Volume	25 mL

A PlasmaQuant® MS with Cetac ASX-560 autosampler was used. The ICP-MS was optimized for maximum sensitivity on Fe isotopes and lowest background on all four Fe isotopes using the integrated Collision Reaction Cell (iCRC) in hydrogen mode. Platinum tipped interface cones were also used to reduce Ni isobaric interferences. The operating conditions are summarized in table 2 . Table 3 shows the potential interferences on Fe isotopes.

Table 2: PlasmaQuant® MS operating conditions

Parameter	Settings
Plasma Gas Flow	7.5 L/min
Auxiliary Gas Flow	1.05 L/min
Nebulizer Gas Flow	0.75 L/min
iCRC Gas Flow	200 mL/min H_2
Plasma RF Power	0.60 kW
Dwell Time	^{52}Cr – 10000 µs ^{54}Fe – 8000 µs ^{56}Fe – 2000 µs ^{57}Fe – 14000 µs ^{58}Fe – 36000 µs ^{60}Ni – 10000 µs
Scans per Replicate	200 (peak hopping, 1pt/peak)
No. of Replicates	15
Pump Rate	15 rpm - black/black PVC pump tubing
Sample Uptake Time	60 s
Stabilization Delay	40 s

Table 3: Fe isotopes, abundance and potential interferences

Isotope	Abundance (%)	Interferences	
		Isobaric	Polyatomic (MO^+/MOH^+)
^{54}Fe	5.85	^{54}Cr (2.37%)	$^{40}Ar^{14}N$
^{56}Fe	91.75		$^{40}Ar^{16}O$; $^{40}Ca^{16}O$
^{57}Fe	2.12		$^{40}Ar^{16}OH$; $^{40}Ca^{16}OH$
^{58}Fe	0.28	^{58}Ni (68.08%)	$^{40}Ar^{18}O$; $^{40}Ca^{18}O$; $^{42}Ca^{16}O$

Samples and Reagents

The following high purity reagents were used for solution preparations:

- Deionized water (>18.2 MΩ.cm, Millipore MiliQ)
- Hydrochloric acid ultra-quality 34% (ROTIPURAN® Ultra)
- Nitric acid supra-quality 69% (ROTIPURAN® Supra)
- Mono-element standard solutions, CertiPUR® 1000 mg/L in 2% HNO_3
- Iron isotopic material: IRMM 634 – 163.61 ± 0.38 µmol/kg of ^{56}Fe in 1.8 M HCl, 4.5 mL; obtained from dissolution of the natural isotopic reference material IRMM-014 (wire) with hydrochloric acid

Calibration standards

Calibration solutions were prepared from high-purity, single-element standards in 1% HNO_3 covering the concentration range from

5 - 50 mg/L for Na and K;
1 - 10 mg/L for Fe;
0.1 - 1 mg/L for Ca, Zn, Mg and Rb;
0.01 - 0.1 mg/L for Cu;
0.001 - 0.050 mg/L for Mn, Ni, V, Cr and Pb.

Sample preparation

Random whole blood samples collected from 6 male, 4 female and 1 child at a medical clinic in Portugal were used for the study. Following digestion, samples were transferred to 25.0 mL polypropylene flasks and made up to volume with 1% HNO_3. Fe isotope ratios were measured following a further 10-fold dilution in 2M HCl of the digested samples. To evaluate the mass bias, IRMM 634 - Fe isotopic standard was matched with two different blood matrix types following matrix characterization.

Results and Discussion

Matrix Characterization

The samples were analyzed for major and minor elements to characterize the matrix prior to accurate determination of the Fe isotope ratios. The purpose of this is to evaluate the concentration range of matrix elements and identify those that may possibly bias the results (eg..Ca, Ni, Cr)

Table 4 shows major and minor elements found in 11 human whole blood samples.

Table 4: Major and minor elements in Whole Blood; values in ppm (n=3)

Ref[a]	Ca	Fe	Na	K	Cu	Zn	Mg	Rb	Mn	Ni	V	Cr	Pb
Child 1	42	359	1425	323	0.58	1.4	39	3.2	0.40	0.11	0.01	0.006	0.39
Male 2	51	286	1629	278	0.86	2.2	27	2.6	0.30	0.11	0.02	0.011	0.29
Male 3	48	320	1506	272	0.74	2.6	27	2.8	0.36	0.11	0.01	0.006	0.31
Male 4	47	365	1447	309	0.42	1.6	34	3.0	0.41	0.11	0.01	0.003	0.30
Male 9	178	319	1567	297	0.89	2.8	29	1.8	0.36	0.14	0.02	0.005	0.27
Male 10	233	441	1594	365	0.94	3.4	40	2.9	0.43	0.12	0.02	0.004	0.23
Male 11	403	262	1718	276	1.1	3.1	68	1.8	0.31	0.14	0.01	0.005	0.26
Female 5	56	292	1713	299	0.61	1.3	32	1.7	0.30	0.11	0.01	0.006	0.25
Female 6	50	335	1530	331	0.53	1.6	31	3.4	0.35	0.11	0.01	0.006	0.23
Female 7	69	332	1757	323	0.74	2.1	40	2.1	0.34	0.11	0.02	0.008	0.30
Female 8	58	236	1899	230	0.91	2.3	36	2.2	0.26	0.13	0.01	0.016	0.27

Majors elements measured in 11 human whole blood samples included Na, Fe, K, Ca and Mg with concentrations ranging from more than 25ppm for Mg to less than 1900ppm for Na . Minor levels of Zn, Rb, Cu and Mn were found and Pb, Ni, V and Cr at trace concentrations. With this information, it was clearly evident that the last three samples (Male 9, 10 and 11) contained higher concentrations of Ca compared to the others.
This characterization identified two matrices (see below) in which to prepare IRMM 634 Fe isotopic standards, in order to determine the influence of matrix effects on Fe isotope measurement accuracy and in determining the instrument mass bias. The main difference being a Ca concentration 5.5 times higher in Matrix 2.

Matrix 1 contained 65 ppm Na, 12 ppm K, 2 ppm Ca and 1.3 ppm Mg based on the mean values of (Child 1, Male 2, Male 3, Male 4, Female 5, Female 6, Female 7 and Female 8) major metals.
Matrix 2 contained 65 ppm Na, 12 ppm K, 11 ppm Ca and 1.3 ppm Mg based on the mean values of (Male 9, Male 10 and Male 11) major metals.

All digested samples were diluted 10-fold prior to the determination of Fe isotope ratios in order to further reduce matrix interferences and approach approximately 2 ppm of Fe in the final solutions, as per the IRMM 634 Fe isotopic standard solutions prepared in both matrix 1 and 2 .

Instrument Mass Bias
The mass bias is a fundamental notion in mass spectrometry and should be taken into account during calculations to obtain good accuracy of measurement. This is a systematic error produced by the instrument. However, the mass bias may be small for heavier ions as they are better focused through the skimmer cone after the sampling cone.

This fractionation coefficient deviation, β, can be defined as a function of the different masses studied. The true ratio of isotopes A and B (R) can be expressed from the ratio measured (r) by different relations called linear law (A.1), exponential (kinetic) law (A.2) or power law (A.3).

The bias per unit mass, α, can be determined by measuring a certified solution or a reference solution when using standard bracketing.

$$\frac{r}{R}=1+\alpha\Delta m \qquad (A.1)$$

$$\beta=\ln\left(\frac{R}{r}\right)/\ln(m_A/m_B) \qquad (A.2)$$

$$R=r\left(\frac{m_A}{m_B}\right)\beta \qquad (A.3)$$

To check the mass bias (Figure 1) and to correct Fe isotope ratios obtained, IRMM 634 was matched with both matrix 1 and 2, bracketing the corresponding sample matrices. The instrument follows the exponential law and both matrices had the same effect on ion transmission.

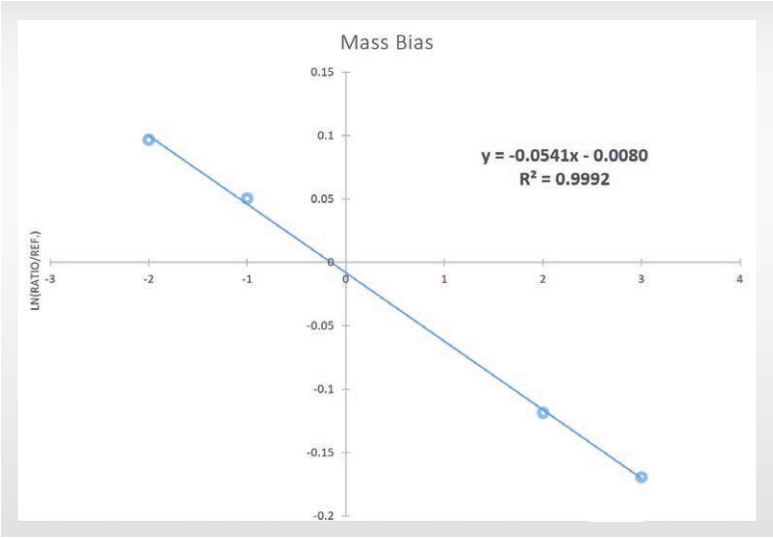

Figure 1: PlasmaQuant® MS mass bias for Fe isotopes obtained in IRMM 634 matched with matrix 1 or 2

Fe isotope ratios

After blank subtraction and mass bias correction, the final Fe isotope ratios in each of the samples were determined and are reported in Table 5.

Table 5: Results of Fe isotope ratios in Human Whole Blood

Ref[a]	$^{54}Fe/^{56}Fe$	$^{57}Fe/^{56}Fe$	$^{58}Fe/^{56}Fe$	$^{54}Fe/^{57}Fe$	$\delta^{56}Fe$	$\delta^{57}Fe$	$\delta^{58}Fe$
Child 1	0.06309	0.02316	0.00306	2.74557	-9.5896	2.9155	-4.9869
Male 2	0.06322	0.02317	0.00306	2.75060	-7.5473	3.1609	-2.9752
Male 3	0.06303	0.02316	0.00306	2.74356	-10.4936	2.7383	-2.1362
Male 4	0.06340	0.02311	0.00306	2.76586	-4.7515	0.4221	-3.3368
Male 9	0.06328	0.02319	0.00304	2.75137	-6.5881	3.8235	-9.1158
Male 10	0.06351	0.02313	0.00307	2.76759	-3.0012	1.6116	-0.6582
Male 11	0.06335	0.02306	0.00305	2.76911	-5.4747	-1.4805	-5.9241
Female 5	0.06298	0.02322	0.00305	2.73409	-11.2877	5.4077	-7.0403
Female 6	0.06300	0.02308	0.00305	2.75178	-11.1019	-0.8779	-8.1287
Female 7	0.06315	0.02308	0.00305	2.75797	-8.7133	-0.7143	-5.5670
Female 8	0.06371	0.02314	0.00303	2.77536	0.0673	1.8350	-12.5166

Variations between isotope ratios are usually small, and therefore they are commonly measured relative to a reference material. Data are commonly presented in the delta-notation (A.4) in parts per thousand (per mil, ‰).

$$\delta^{56}Fe_{IRMM14} = \left(\frac{^{56}Fe/^{54}Fe_{Sample}}{^{56}Fe/^{54}Fe_{IRMM14}} - 1 \right) x1000 \quad (A.4)$$

with $^{56}Fe/^{54}Fe_{Sample}$ being the Fe isotope ratio in the samples and $^{56}Fe/^{54}Fe_{IRM634}$ the isotope ratios in the reference material. For Fe, data are commonly reported relative to IRMM-014, and has been done so here for consistency as IRMM 634 is a certified solution made up from IRMM-014 wire.

The following isotopic diagrams (Figure 2) clearly shows a distinct difference between male and female Fe isotope ratios in whole blood.

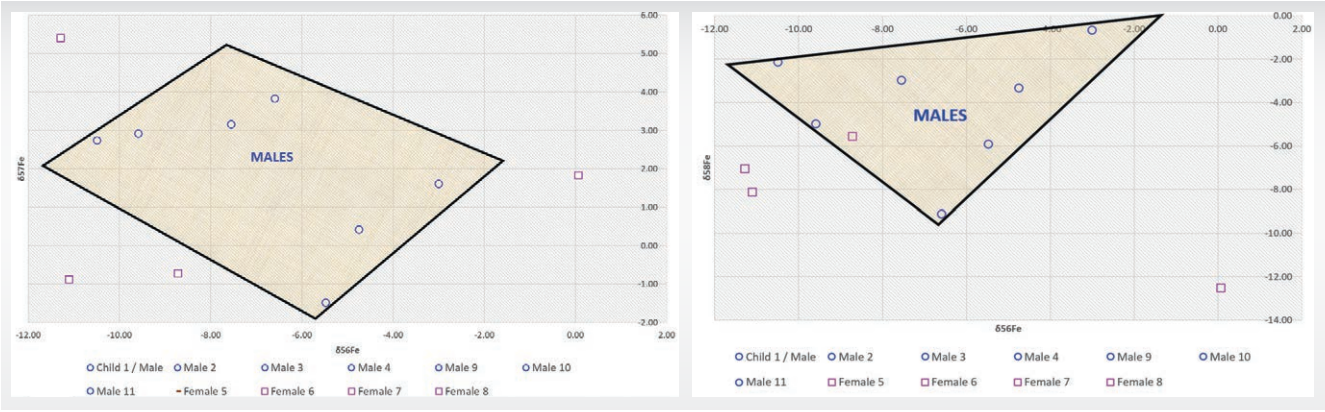

Figure 2: Isotopic composition of Fe in whole blood. Fe isotope ratios are presented on a δ-scale ($\delta^{57}Fe$ at left and $\delta^{58}Fe$ at right versus $\delta^{56}Fe$). More negative δ-values correspond to a lower $^{56}Fe/^{54}Fe$ isotope ratio, i.e., an enrichment in the lighter Fe isotopes, compared to the reference material of non-biological origin (IRMM-014)

Iron in blood of females showed δ-values more negative, meaning it's less enriched in the lighter Fe isotopes than the blood of males.These values are in accordance to previous studies. Men and women differ on average in the Fe isotope composition of their blood with women having more of the heavier Fe isotopes in blood than men. In terms of age group (Child 1 versus Males) the differences are insignificant.

Conclusion

A method was developed for the preparation and determination of Fe isotope ratios in 11 human whole blood sample by ICP-MS with the precision and accuracy to clearly distinguish between female and male samples. Polyatomic interferences on Fe isotopes were reduced to negligible levels using cool plasma conditions with only 9.3 L/min of argon gas and hydrogen as a reaction gas in the patented iCRC. Platinum tipped cones provided lower Ni intensities.

Following matrix characterization, samples were diluted to adjust Fe concentrations to approximately 2ppm. Matching the acid molarity and matrix between samples and the reference material (IRMM634) was strategic to achieving accurate measurements. Accuracy of isotope ratio measurements were not sensitive to higher calcium concentrations when comparing matrix 1 and 2, as both matrices lead to the same mass bias.

Measurements of ~2 ppm Fe in the final sample/standard solutions were repeated 15 times, with Fe isotope ratio precisions of between 0.10 – 0.50 (RSD%) being determined.

References

Vanhaecke F, Balcaen L, De Wannemacker G, Moens L (2002) Capabilities of inductively coupled plasma mass spectrometry for the measurements of Fe isotope ratios. J Anal At Spectrom 17:933–943

Headquarters
Analytik Jena AG
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

en · 11/2016 | **For Research Use Only**