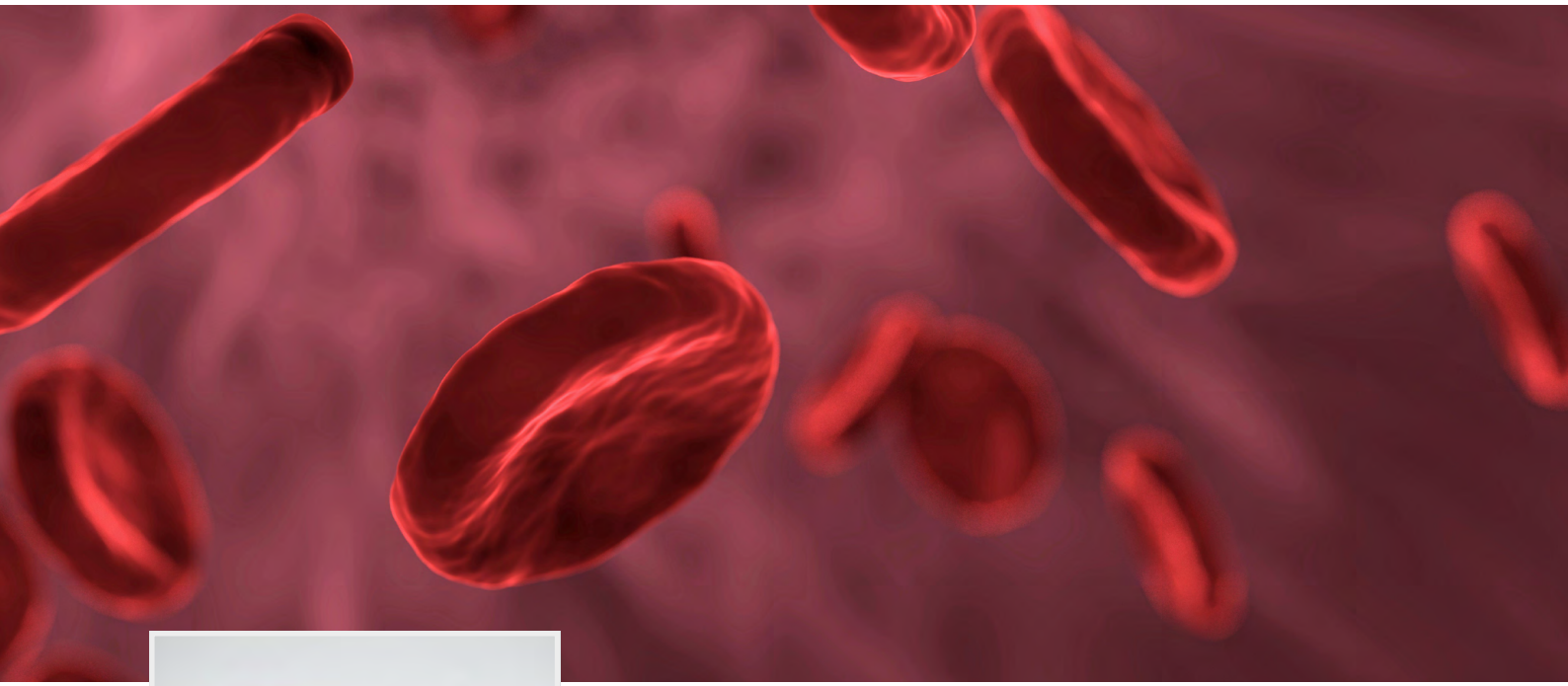


Challenge

Accurate quantification of the metallic profile in whole blood with a robust method ensuring lowest LODs for elements which are typically affected by strong interferences.

Solution

The BOOST technology of the PlasmaQuant MS Q enables the accurate measurement of lowest quantities of chromium and other elements in whole blood.

BOOSTing Limits of Detection for Chromium and Other Trace Elements in Whole Blood

Introduction

The blood circulatory system is a transport system which continuously supplies the body with the oxygen, vitamins, fatty acids and nutrients it needs. With iron being the most prominent example, many elements are dissolved in blood or embedded in proteins that are required for the proper functioning of the organism. The presence of too high or too low concentrations of these elements can be an indication of a deficiency or an intoxication potentially leading to severe diseases. For this reason, the metallic profile of biological samples such as blood, serum, plasma and urine is routinely analyzed. In recent years, inductively coupled plasma mass spectrometry (ICP-MS) has become the method of choice for analyzing these sample types because of its speed and low detection limits.[1]

Whole blood is a complex sample matrix with solid and liquid components and a relatively high carbon content deriving from the proteins and lipids in the blood. In some cases it is sufficient to determine the elemental concentration in the serum. However, some elements are partially located in the non-soluble blood fraction, the hematocrit, making the analysis of whole blood necessary. Due to its solid and liquid components the sample preparation of whole blood samples is difficult, as solid components must not precipitate. For this reason different procedures for sample preparation have been developed.[2]

Here, we present a robust method for obtaining a metallic profile of whole blood with concentrations ranging from ppt to ppm level using alkali dilution for easy and fast sample preparation. With the patented BOOST technology, elements

such as As, Se, Cr and V, which are typically difficult to measure in whole blood with ICP-MS, can be analyzed correctly with method limits of detection in the ng/l range. The accurate quantification of all elements during a long-term measurement of 8 hours proved the applicability of the method for the routine analysis of whole blood.

Materials and Methods

A PlasmaQuant MS Q equipped with ASPQ3300 autosampler, platinum cones, Micro Mist (0.4 ml/min) nebulizer, Scott double-pass spray chamber and 2.4 mm injector torch was used for the analysis.

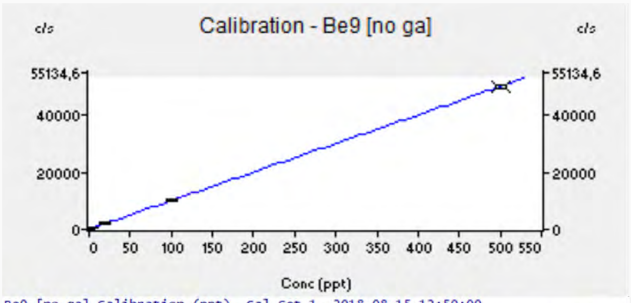
Samples and Reagents

- All samples and standards were prepared using high-purity reagents. A matrix-matched solution was used for calibration and sample dilution.
- To stabilize the solid components of the blood and to mimic the carbon content this solution contained deionized water <0.055 mS (ELGA Lab), 2% NH_4OH, (Sigma-Aldrich), 2% isopropanol (Honeywell), 0.1% TritonX100 (Merck).
- All samples were diluted by a factor of 20.
- Calibration solutions were prepared from a stock solution using single-element standards of the individual elements. The concentrations used for calibration are shown in Table 1.
- All experiments were performed under non-clean room conditions to assess the robustness of the method for a standard laboratory environment.

Table 1: Concentration of the standards used for calibration.

Element	Unit	Standard 1	Standard 2	Standard 3	Standard 4
Be	ng/l	4	20	100	500
Al	ng/l	40	200	1000	5000
V	ng/l	8	40	200	1000
Cr	ng/l	20	100	500	2500
Mn	ng/l	40	200	1000	5000
Co	ng/l	4	20	100	500
Ni	ng/l	8	40	200	1000
Cu	ng/l	4	20	100	500
Zn	ng/l	16	80	400	2000
As	ng/l	8	40	200	1000
Se	ng/l	160	800	4000	20000
Mo	ng/l	4	20	100	500
Pd	ng/l	8	40	200	1000
Cd	ng/l	4	20	100	500
Sn	ng/l	–	–	100	500
I	ng/l	80	400	2000	10000
Pt	ng/l	8	40	200	1000
Tl	ng/l	8	40	200	1000
Pb	ng/l	160	800	4000	20000
Bi	ng/l	–	20	100	500

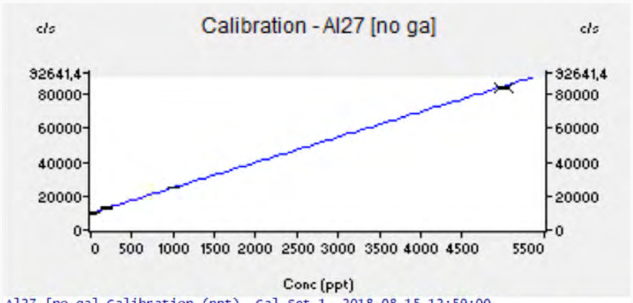
Out of the 20 analytes, six representative calibration curves (Be, Al, Cr, Se, Cd and Tl) are shown in Figure 1. Correlation coefficients >0.9999 were achieved for all elements. The obtained correlation coefficients, low RSDs and minor deviations of the individual standards from the regression curve show the excellent quality of the calibration under non-clean room conditions even at low ng/l concentration and in a matrix-matched solution.



Be9 [no ga] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	50	21,2	42,810,0000	-0,0231	-0,0231		-
Standard 1	474	53,4	11,254,0000	4,1533	0,1533	3,83	
Standard 2	2029	156,1	7,69 20,0000	19,7065	-0,2935	-1,47	
Standard 3	10232	289,6	2,83 100,0000	100,9794	0,9794	0,98	
Standard 4	50396	712,6	1,41 500,0000	498,8398	-1,1602	-0,23	

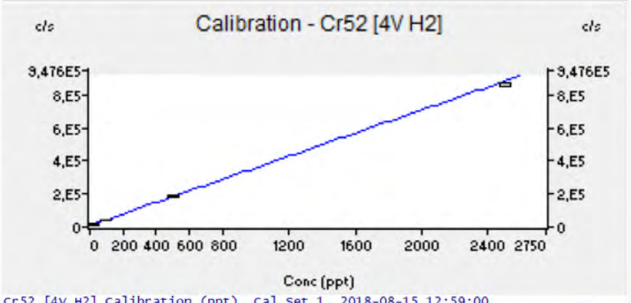
Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 0,999996
Limit of detection: 0,6357 ppt
Limit of quantitation: 2,1189 ppt
Blank Equivalent Concentration: 0,4950 ppt
Calibration Equation: c/s = (49,6 + 2,314 + 100,4*conc)*[I/S Ratio]



Al27 [no ga] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	9745	203,2	2,08 0,0000		-14,8698	-14,8698	-
Standard 1	10719	246,1	2,30 40,0000		41,8094	1,8094	4,52
Standard 2	12945	168,3	1,30 200,0000		199,7117	-0,2883	-0,14
Standard 3	25170	161,8	0,64 1000,000		1011,144	11,1443	1,11
Standard 4	84679	555,1	0,66 5000,000		4973,828	-26,1719	-0,52

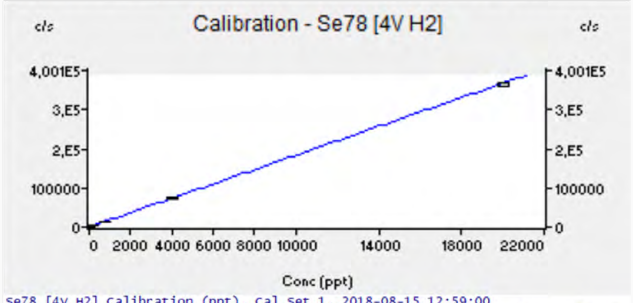
Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 0,999987
Limit of detection: 28,8133 ppt
Limit of quantitation: 96,0443 ppt
Blank Equivalent Concentration: 460,6632 ppt
Calibration Equation: c/s = (9744,7 + 222,0 + 14,93*conc)*[I/S Ratio]



Cr52 [4V H2] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	9158	235,3	2,57 0,0000		-0,4146	-0,4146	-
Standard 1	16377	572,4	3,50 20,0000		20,2385	0,2385	1,19
Standard 2	45241	645,7	1,43 100,0000		102,9061	2,9061	2,91
Standard 3	185669	2294,4	1,24 500,0000		500,4829	0,4829	0,10
Standard 4	872699	14224,8	1,63 2500,000		2438,691	-61,3086	-2,45

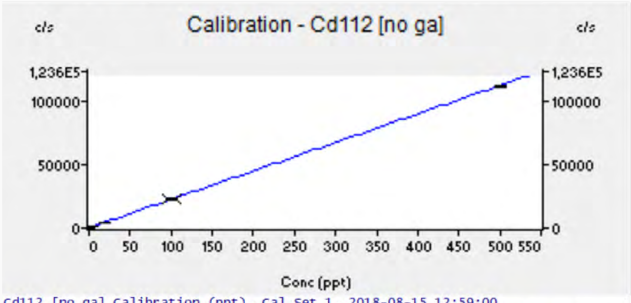
Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 0,999986
Limit of detection: 2,0167 ppt
Limit of quantitation: 6,7223 ppt
Blank Equivalent Concentration: 26,1601 ppt
Calibration Equation: c/s = (9157,7 + 144,9 + 349,4*conc)*[I/S Ratio]



Se78 [4V H2] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	103	14,9	14,480,0000		-0,0739	-0,0739	-
Standard 1	3067	78,7	2,57 160,0000		160,1334	0,1334	0,08
Standard 2	14992	214,4	1,43 800,0000		815,6337	15,6337	1,95
Standard 3	73771	1040,5	1,41 4000,000		3980,014	-19,9858	-0,50
Standard 4	367415	4453,1	1,21 20000,00		19800,03	-199,9746	-1,00

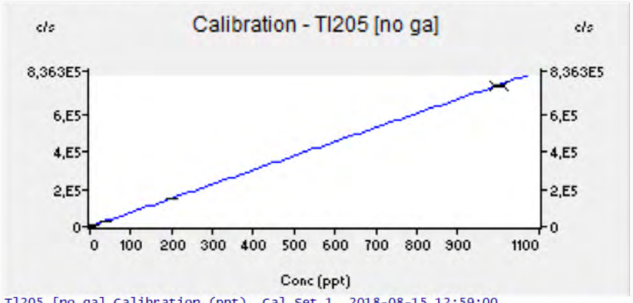
Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 0,999999
Limit of detection: 2,4299 ppt
Limit of quantitation: 8,0995 ppt
Blank Equivalent Concentration: 5,5938 ppt
Calibration Equation: c/s = (102,9 + 1,358 + 18,36*conc)*[I/S Ratio]



Cd112 [no ga] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	76	86,7	114,30,0000		0,0354	0,0354	-
Standard 1	941	111,9	11,894,0000		3,8494	-0,1506	-3,77
Standard 2	4585	142,1	3,10 20,0000		19,9972	-0,0028	-0,01
Standard 3	22972	329,5	1,43 100,0000		100,9987	0,9987	1,00
Standard 4	113711	657,4	0,58 500,0000		499,2490	-0,7510	-0,15

Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 0,999997
Limit of detection: 1,1582 ppt
Limit of quantitation: 3,8605 ppt
Blank Equivalent Concentration: 0,3378 ppt
Calibration Equation: c/s = (75,8 - 7,968 + 224,8*conc)*[I/S Ratio]



Tl205 [no ga] Calibration (ppt), Cal Set 1, 2018-08-15 12:59:00

Soln Type	c/s	SD(c/s)	%RSD	Std Conc	Calc Conc	Error	%Error
Blank	95	39,4	41,340,0000		-0,0018	-0,0018	-
Standard 1	6249	264,2	4,23 8,0000		8,0931	0,0931	1,16
Standard 2	30451	381,1	1,25 40,0000		39,9658	-0,0342	-0,09
Standard 3	152809	2383,1	1,56 200,0000		200,3748	0,3748	0,19
Standard 4	761848	4935,8	0,65 1000,000		999,6477	-0,3523	-0,04

Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
Correlation Coefficient: 1,000000
Limit of detection: 0,1555 ppt
Limit of quantitation: 0,5185 ppt
Blank Equivalent Concentration: 0,1254 ppt
Calibration Equation: c/s = (95,3 + 1,357 + 760,4*conc)*[I/S Ratio]

Figure 1: Representative calibration curves.

Instrument settings and method parameters

Elements which are not subject to polyatomic interferences were measured in no gas mode. Argon-based interferences were removed by injecting hydrogen as a reaction gas via the integrated collision reaction cell (iCRC). The high carbon content of the whole blood matrix results in the formation of $^{40}Ar^{12}C^+$ species, which directly interfere with the most sensitive isotope of Chromium, ^{52}Cr. To achieve highest sensitivity and lowest limits of detection for elements measured in reaction gas mode, the patented BOOST technology was used. In BOOST mode, a positive voltage is applied to the skimmer cone allowing to compensate for the loss in sensitivity when using reaction gases.
Internal standards (Li, Rh and Ir) were added online to the sample via a Y-piece at 10 µg/l concentration. The method parameters used are listed in Table 2.

Table 2: Method parameters.

Parameter	Specification
Plasma Gas Flow	9 l/min
Auxiliary Gas Flow	1.50 l/min
Nebulizer Gas Flow	0.98 l/min
Spray Chamber Temperature	3 °C
RF Power	1400 W
Sampling Depth	5.0 mm
Dwell Time	10 ms (50 ms for Al, V, Cr, Se)
Scans per Replicate	15 (peak hopping, 1 pt/peak)
No. of Replicates	7
Pump Rate, Tubings	15 rpm, black/black PVC pump tubing for sample, orange/green PCV tubing for internal standards
iCRC Gas Flow	H_2, 200 ml/min (V, Cr, As, Se)
BOOST voltage (for V, Cr, As, Se)	10 V

Results and Discussion

Depending on the sample matrix, certain elements are interfered by polyatomic molecules having a mass-to-charge ratio identical to the analyte. This results in an analyte concentration that is apparently too high unless the interference is removed. Polyatomic interferences can be divided into those which can be removed by kinetic energy discrimination (physical process) and those which can be removed by a chemical reaction. Typically, argon-based interferences are removed by injecting a reactive gas, such as hydrogen, into a collision-reaction cell. Elements and interfering polyatomic molecules which are typically formed in a blood matrix are listed in Table 3.

Table 3: Analyte ions and corresponding polyatomic interferences typically formed in blood.

Analyte ion	$^{51}V^+$	$^{52}Cr^+$	$^{75}As^+$	$^{78}Se^+$
Polyatomic interferences	$^{38}Ar^{13}C^+$, $^{35}Cl^{16}O^+$	$^{40}Ar^{12}C^+$, $^{36}Ar^{16}O^+$, $^{38}Ar^{14}N^+$	$^{40}Ar^{35}Cl^+$, $^{36}Ar^{39}K^+$, $^{38}Ar^{37}Cl^+$	$^{38}Ar^{40}Ar^+$, $^{38}Ar^{40}Ca^+$

In conventional ICP mass spectrometers, the collision-reaction cell for interference removal is directly located in front of the quadrupole. The PlasmaQuant MS family with a collision cell being integrated in the skimmer cone (integrated collision-reaction cell, iCRC) is the only ICP-MS on the market being capable of removing interfering polyatomic molecules directly after they are formed and before they can enter the ion optics. Similar to conventional collision reaction cells, gas is injected into the iCRC for interference removal.

The presence of reactive gas molecules in the ion path results in collisions of gas molecules and analyte ions. As a consequence, the kinetic energy of the analyte ions is decreased, reducing the fraction which can be extracted from the extraction lenses. This directly reduces the sensitivity for those elements. Using the patented BOOST technology, a positive voltage is applied to the skimmer cone. The analyte ions are then reaccelerated uniformly which greatly improves sensitivity. Therefore, the BOOST technology directly leads to better limits of detection for interfered elements.

The background equivalent concentration (BEC), limits of detection (LOD) and limits of quantification (LOQ) were determined to assess the performance of the method. Instrument detection limits (IDL) were calculated performing a noise analysis of the measured blank solution (three sigma method). The obtained LODs and LOQs were converted into method detection limits (MDL) and method quantification limits (MQL) by multiplying them by a factor of 20 as used for sample dilution.

Table 4: Determined instrument limit of detection (IDL) and quantification (IQL) and resulting method limit of detection (MDL) and quantification (MQL) for elements in whole blood.

Isotope	IDL [ng/l]	IQL [ng/l]	BEC [ng/l]	MDL [µg/l]	MQL [µg/l]
^{9}Be	0.64	2.12	0.50	0.01	0.04
^{27}Al	28.8	96.0	461	0.58	1.92
^{51}V	2.57	8.58	2.17	0.05	0.17
^{52}Cr	2.02	6.72	26.2	0.04	0.13
^{55}Mn	10.2	34.1	54.5	0.20	0.68
^{59}Co	1.30	4.34	2.40	0.03	0.09
^{60}Ni	3.96	13.2	15.2	0.08	0.26
^{65}Cu	19.9	66.3	27.0	0.40	1.33
^{66}Zn	72.6	242	455	1.45	4.84
^{75}As	1.70	5.67	3.24	0.03	0.11
^{78}Se	2.43	8.09	5.59	0.05	0.16
^{98}Mo	1.16	3.85	1.36	0.02	0.08
^{105}Pd	0.60	2.00	1.19	0.01	0.04
^{112}Cd	1.16	3.86	0.34	0.02	0.08
^{119}Sn	5.49	18.3	26.2	0.11	0.37
^{127}I	20.4	67.9	58.5	0.41	1.36
^{195}Pt	0.61	2.02	1.12	0.01	0.04
^{205}Tl	0.16	0.52	0.13	0.003	0.01
$^{206\text{-}208}$Pb	1.44	4.80	2.01	0.03	0.10
^{209}Bi	0.36	1.20	3.27	0.007	0.02

Instrument detection limits in the low ppt range were obtained for all elements being the prerequisite for the analysis of trace concentrations in whole blood. The IDLs listed in Table 4 were determined for this specific method, matrix and calibration. For a different matrix or method, by using chemicals having a higher purity or by working under clean room conditions, the LODs can be improved.

To prove the accuracy of the method, certified reference materials (CRMs) and human whole blood samples spiked with a known concentration of analyte were measured. The following elemental concentrations in human blood and in the certified reference materials (CRMs) were measured (Table 5). The reference concentration (5-95% percentile) of human blood and the confidence interval of the CRMs is written in brackets.[3-5]

Table 5: Determined concentrations of human whole blood and certified reference materials with their certified/reference concentration in brackets.

Isotope	Seronorm lvl.2 LOT 1406264 [µg/l]	ClinChek® LOT1077 Level I [µg/l]	ClinChek® LOT1077 Level II [µg/l]	ClinChek® LOT1077 Level IIII [µg/l]	Whole blood [µg/l]
^{9}Be	5.92 (4.13-6.22)	<MDL (n. s.)	<MDL (n. s.)	<MDL (n. s.)	<MDL (<0.03)
^{27}Al	77.5 (55.0-82.7)	10.1 (n. s.)	15.6 (n. s.)	26.8 (n. s.)	4.28 (4.9-14.9)
^{51}V	5.27 (3.98-5.98)	<MQL (n. s.)	<MDL (n. s.)	<MDL (n. s.)	<MQL (<0.35)
^{52}Cr	11.2 (8.44-12.7)	1.66 (1.03-1.72)	6.08 (4.20-7.01)	12.6 (8.44-12.7)	0.66 (<0.87)
^{55}Mn	34.5 (25.1-37.7)	9.49 (7.09-10.6)	16.3 (12.3-18.5)	23.9 (17.7-26.5)	8.85 (5.9-13.3)
^{59}Co	5.62 (4.13-6.22)	1.73 (1.24-1.87)	7.11 (5.70-8.55)	13.5 (10.4-15.7)	0.57 (0.2-0.63)
^{60}Ni	16.3 (12.7-19.1)	2.21 (1.41-2.35)	5.03 (3.74-5.61)	13.3 (10.3-15.5)	1.49 (<1.36)
^{65}Cu	1302 (1070-1600)	711 (542-813)	1111 (885-1330)	1695 (1340-2010)	840 (743-1513)
^{66}Zn	8361 (5700-8500)	4866 (3670-5500)	6580 (5020-7530)	8630 (6390-9580)	4955 (4220-7198)
^{75}As	13.3 (11.3-17.0)	5.91 (4.34-6.51)	10.6 (7.97-12.0)	20.9 (15.5-23.2)	0.66 (0.5-4.2)
^{78}Se	159 (128-193)	81.7 (60.2-90.3)	129 (100-150)	172 (134-201)	109 (85-142)
^{98}Mo	5.40 (4.24-6.37)	2.40 (1.65-2.48)	4.96 (3.58-5.37)	9.80 (6.97-10.5)	0.60 (0.26-0.77)
^{105}Pd	0.09 (n. s.)	0.84 (0.578-0.867)	2.01 (1.34-2.01)	3.81 (3.38-5.01)	0.05 (<0.05)
^{112}Cd	5.54 (4.00-6.02)	1.32 (0.987-1.48)	2.94 (2.30-3.45)	6.76 (5.06-7.59)	0.35 (0.13-1.82)
^{119}Sn	4.79 (4.19-6.30)	1.96 (1.73-2.60)	4.20 (3.87-5.81)	7.91 (7.34-11.0)	<MQL (0.14-0.64)
^{127}I	99.2 (86-129)	-	-	-	35.5 (35-118)
^{195}Pt	<MQL (0.0053*)	1.82 (1.34-2.00)	2.67 (1.95-2.92)	5.33 (3.97-5.95)	<MDL (<0.008)
^{205}Tl	9.09 (8.01-12.2)	0.66 (0.656-0.984)	3.52 (3.35-5.81)	7.15 (6.70-10.1)	0.03 (0.004-0.034)
$^{206\text{-}208}$Pb	333 (269-405)	51.0 (43.6-65.3)	193 (176-263)	403 (340-510)	10.8 (5.4-39.3)
^{209}Bi	5.66 (3.99-6.00)	<MDL (n. s.)	<MDL (n. s.)	<MDL (n. s.)	<MDL (<0.03)

* approximate value, not certified, n. s.: not specified

The metallic profile of whole blood was determined accurately using the PlasmaQuant MS Q and its patented integrated BOOST technology and collision reaction cell (iCRC) for interference removal. To mimic a measurement of blood samples from different subjects, the sample introduction system was rinsed after each analysis. Blood samples were measured first, followed by the measurement of matrix spikes and a blank solution to investigate spike recovery and washout behavior. The concentrations measured in the certified reference materials match the specification. The elemental concentration in the whole blood sample was within the 5–95% percentile of the reference range of human blood proving the accuracy of the method. Furthermore, matrix spikes of human whole blood, to which a known amount of analyte was added, were measured for 8 hours to demonstrate the robustness of the method and the instrument. To be able to precisely measure the matrix spike, the amount of added analyte was chosen to be equal or greater than the elemental concentration in the sample. For addition, 10% of either calibration standard 3 or calibration standard 4 were added to the blood sample. Stable and correct recovery rates of the matrix spike were measured over 8 hours covering an entire working day (Figure 2).

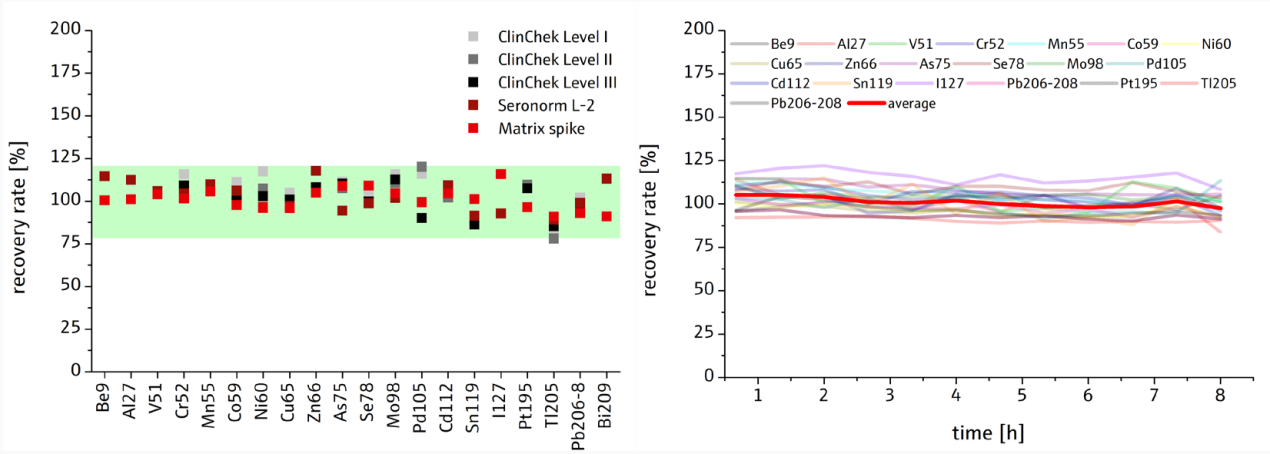


Figure 2: Recovery rates of the certified reference materials (CRMs) and matrix spikes as a function of the element analyzed (left) and as a function of time (right). The measured concentrations of the CRMs and the matrix spike were within the specified uncertainty (80–120%, highlighted in green). The matrix spike was accurately measured over 8 hours proving the robustness of the method and the instrument.

The application of the BOOST technology in combination with the injection of hydrogen as reaction gas proved to be very effective for the measurement of V, Cr, As and Se as demonstrated by the accuracy of the obtained results and the achievable limits of detection in the ng/l range. Only minor deviations of the determined concentrations in human whole blood were measured during the long-term run for 8 hours (Figure 3). Precise and accurate measurements of human blood were performed over 8 hours covering a typical day of work proving the robustness of the method and the instrument.

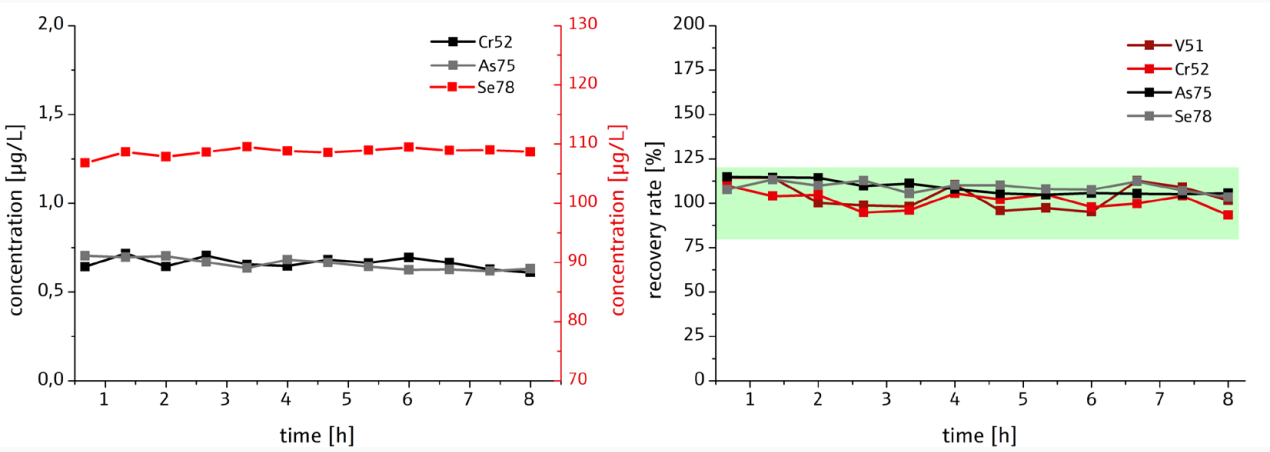


Figure 3: Concentration of elements which are typically difficult to determine in whole blood as a function of time (left). Over 8 hours only minor deviations of the elemental concentration were detected. The matrix spike showed excellent recovery rates during the entire measurement period (right). All recovery rates were within 80–120% (highlighted in green) and the concentration of the whole blood samples was within the reference range of human blood. The concentration of vanadium in the blood sample was below the MQL and is not shown. The added concentration of vanadium in the matrix spike was accurately quantified.

Conclusion

During the last years, the importance of trace elements in biological samples has become evident as the metallic profile can be used to predict diseases or indicate deficiencies. However, biological samples such as blood are typically difficult to analyze due to their complex sample matrix. Using the PlasmaQuant MS Q featuring the patented BOOST technology, the metallic profile of blood can be accurately measured with method limits of detection in the ng/l range. The all-digital detector avoids inaccurate and time-consuming cross calibrations with its attenuation function allowing to measure elements from ppt to ppm range. The integrated collision and reaction cell in combination with the BOOST functionality enables the measurement of strongly interfered elements, such as Cr in whole blood, with high accuracy, precision and sensitivity in a routine fashion. Lowest limits of detection, method robustness, cost-efficient operation by its low argon consumption and the intuitive software with built-in quality controls make the PlasmaQuant MS family the ideal solution for the routine analysis of biological samples.

References

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