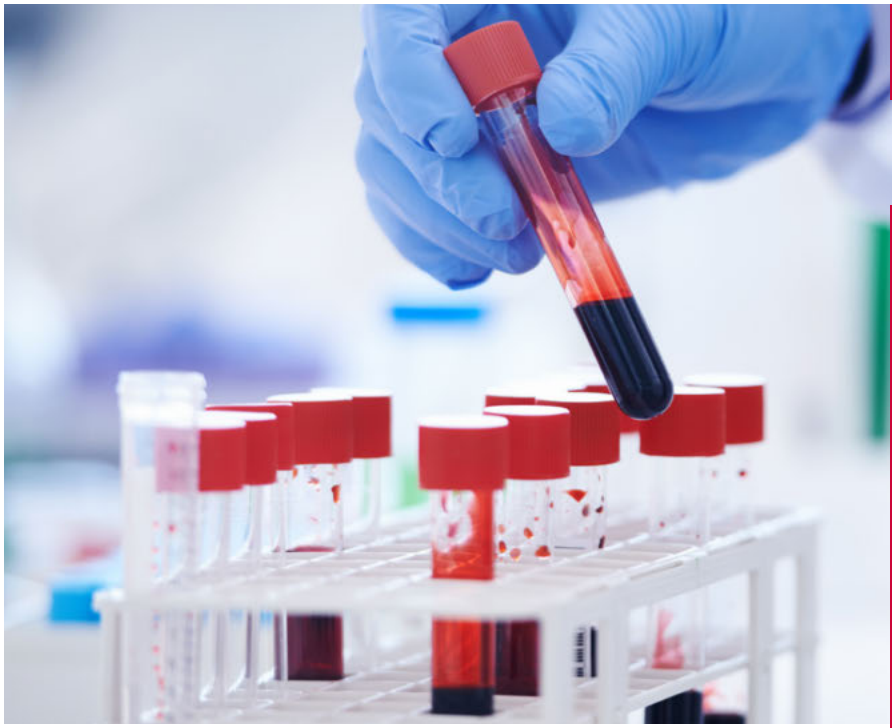

APPLICATION NOTE

ICP - Mass Spectrometry

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Analysis of Blood Using NexION 5000 ICP-MS

Introduction

For many years, inductively coupled plasma mass spectrometry (ICP-MS) has been the tool of choice for the trace analysis of elements like lead (Pb), arsenic (As), mercury (Hg), and copper (Cu) in bodily fluids such as urine, blood, serum and saliva, as well as in tissues. Single elements or panels of toxic and nutritional elements are run in these matrices, providing doctors with insights into the patient's condition. Researchers have found correlations between essential element levels and diseases, metabolic disorders, environmental exposures, and nutrition. Owing to the increased popularity of orthopedic implants, elements like titanium (Ti) and cobalt (Co) have also been added to the list of commonly tested analytes. Although these elements are not classified as either essential or toxic, they can give medical providers information on the implant's degradation.

Blood and serum are two common biological fluids which present challenges for trace metal analysis. Blood is a complex mixture, composed mostly of water, but also contains proteins, glucose, mineral salts, hormones, as well as red and white blood cells. Serum is derived from blood and has a similar composition, although it does not contain red or white blood cells or fibrinogens.

PerkinElmer's NexION® 5000 multi-quadrupole ICP-MS has a winning combination of reaction and collision capabilities with triple quadrupole technology for spectral interference removal.[1] This design allows for the accurate determination of low and high levels of analytes in one analytical run. Using a simple sample preparation technique based upon dilution with an appropriate diluent, it is possible to quickly and precisely measure panels or individual analytes in challenging biological matrices.

This application note summarizes results of blood analysis, highlighting the NexION 5000 ICP-MS' technological advantages for this application.

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Experimental

Instrumental Conditions

All analyses were carried out on the NexION 5000 ICP-MS in a standard configuration with the instrumental conditions and parameters shown in Table 1.

To increase sample throughput, a high-productivity 7-port valve with a 1 mL sample loop was used in flow-switching mode.

Table 1. Instrumental Parameters.

Parameter	Description / Value
Sample Uptake Rate	Pumped at ~200 µL/min
Nebulizer	PFA ST
Spray Chamber	SilQ™ cyclonic
Torch	One-piece SilQ with 2 mm injector
RF Power	1600 W
Cones	Pt-tip Sampler and Skimmer, Ni Hyper-skimmer
Reaction Gases	Ammonia and Oxygen (100%)
MS/MS and Mass Shift Modes	Q1 and Q3 operating at 0.7 amu resolution

Standards and Sample Preparation

To determine the accuracy of the methodology, reference material ClinChek® Blood Control (Recipe Chemicals and Instruments GmbH, Munich, Germany) was used. Three levels of the reference material (normal, elevated and high) were analyzed.

Sample preparation for blood matrices using acidic and basic diluents was discussed elsewhere.[2] In this work, the 50x dilution with an acidic diluent was used. The acidic diluent was a mixture of 0.5% HNO_3 (Optima Grade, Thermo Fisher Scientific, Waltham, Massachusetts, USA) + 0.05% Triton™ X (Sigma-Aldrich™, St. Louis, Missouri, USA), (all v/v) + 0.25 mg/L gold (Au). Although nitric acid was required to keep elements stable in solution, a low concentration was used to prevent precipitation of proteins and cell debris. Triton™ X was used to help rinse the spray chamber and to aid the solubilization of proteins and cell membranes, while the addition of gold aided mercury stability and washout.

Carrier and rinse solutions were the same as the diluent used to prepare samples and standards. Internal standards (2 ppb of Rh and Ir) were also prepared in the same diluent and mixed on-line with the sample stream in the switching valve.

Calibration standards were prepared by diluting ready-to-use blood standard with the diluent (50x, 100x and 200x). This matrix-matched calibration standard consisted of lyophilized blood with the addition of commonly analyzed elements of known concentrations. The benefit of this approach was to eliminate the need to collect pooled-blood samples to perform matrix-matched calibrations.

For optimal performance, analyses were run in Focusing mode with MS/MS or Mass Shift conditions. Reaction mode with NH_3, or O_2 is the most effective way of removing spectral interferences by changing them into other species of a different mass (MS/MS), or by creating cluster ions with the analyte (Mass Shift). In MS/MS mode, Q1 and Q3 are set up at the same mass, while in Mass Shift mode, an analyte is measured at a higher mass than the parent ion as an ion product with a reaction gas.

The ability to run up to four different cell gases, as well as run in Standard mode, within the same method and from a single optimization file is a powerful feature of the NexION 5000 system. This approach results in enhanced ease-of-use and efficient interference removal. All analytes could be measured within a single analytical run, in the same method without the need to divide them into separate panels.

Results and Discussion

Since there are no specific regulatory requirements for trace-elemental analysis in blood, the elements measured and reported often vary between laboratories. In some cases, specific panels of elements are analyzed, while in others, the analyses may call for individual elements (such as Pb in blood). As a result, the elements chosen for this work represent a typical cross-section of those routinely analyzed in blood.

The main benefit of using the triple quadrupole capability of the NexION 5000 is the ability to reject ions that are not the analytes of interest by the Q1 quadrupole and the possibility of using not only MS/MS but also Mass Shift mode. The use of the Universal Cell along with four cell gases on the NexION 5000 ICP-MS allows the optimal conditions to be chosen for each analyte, whether it is a Reaction, Collision, or Standard mode. In Reaction mode, ammonia is the most effective cell gas for eliminating the argon-, carbon- and chloride-based interferences on chromium, manganese and other elements, delivering single-ppt detection limits for these elements in biological matrices. On the other hand, oxygen is the most efficient reaction gas for eliminating the metal-oxide interferences on cadmium (MoO^+) and mercury (WO^+) by changing interferences into different oxide ions. Also, O_2 reacts and creates clusters with some elements like P, Sc, As and Se, allowing the application of Mass Shift to measure those elements as oxide ions at masses where spectral interferences do not exist. For high mass analytes with no polyatomic interferences in blood, Standard mode is preferred.

In Figure 1, a Q3 scan (Single Quad Mode) of a 100 ppt multi-element standard from mass 75 to 92 is shown while O_2 was introduced into the Universal Cell. In this mode, the Q1 quadrupole acts as ion guide transmitting all masses. The analyzed standard is a mixture of 50 elements including Ge, Zr and diluted HCl. Multiple peaks from components of this standard are displayed and a peak formed by AsO^+ is present at mass 91.

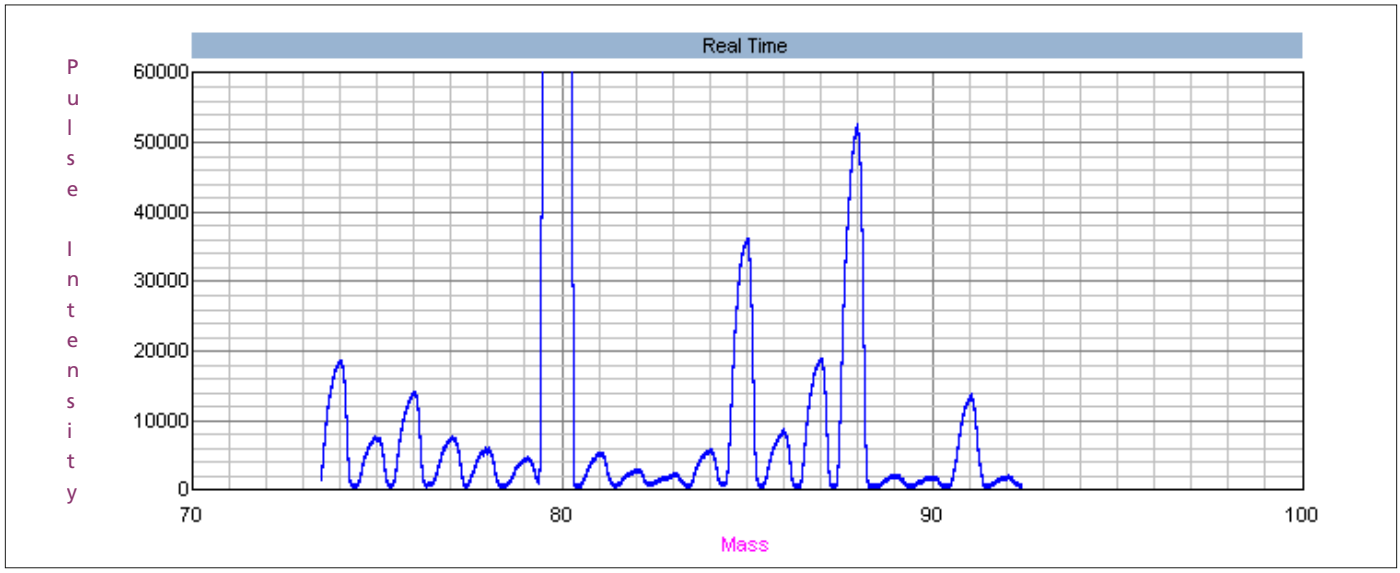

Figure 1. Q3 scan (Single Quad mode) of a 100-ppt mixed-element standard from mass 75 to 92.

The same mixed-element standard solution was scanned in Product Ion Scan mode with O_2, while instead of Q1 transmitting all ions, it was set to 0.7 amu resolution at mass 75. Figure 2 shows a clean background with only two peaks – a small one at mass 75 (CoO^+ and $ArCl^+$) and a much larger one at mass 91, arsenic shown as AsO^+. Arsenic is measured in Mass Shift mode after a reaction with O_2 without any worry about potential interferences from Ge, Zr and Cl.

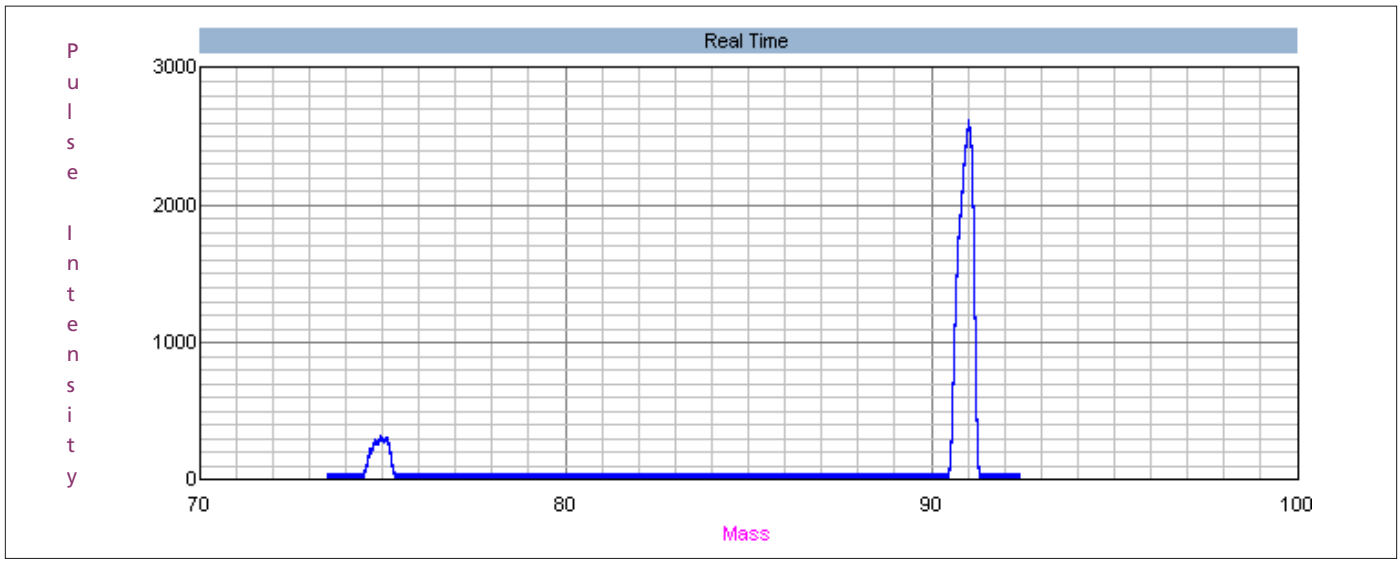

Figure 2. Product Ion scan of a 100-ppt mixed-element standard from mass 75 to 92 while Q1 transmits only ions at mass 75.

The same mixed-element standard solution was scanned in Product Ion Scan mode with O_2, while Q1 was set to a resolution of 0.7 amu set at mass 80. The plot in Figure 3 shows a clean background with a peak at mass 96. Se 80^+ reacts with O_2 and creates a SeO^+ cluster at mass 96. Therefore, Se can be measured in Mass Shift mode after reaction with O_2 without potential interferences from Zr, Mo and Ru residing at mass 96.

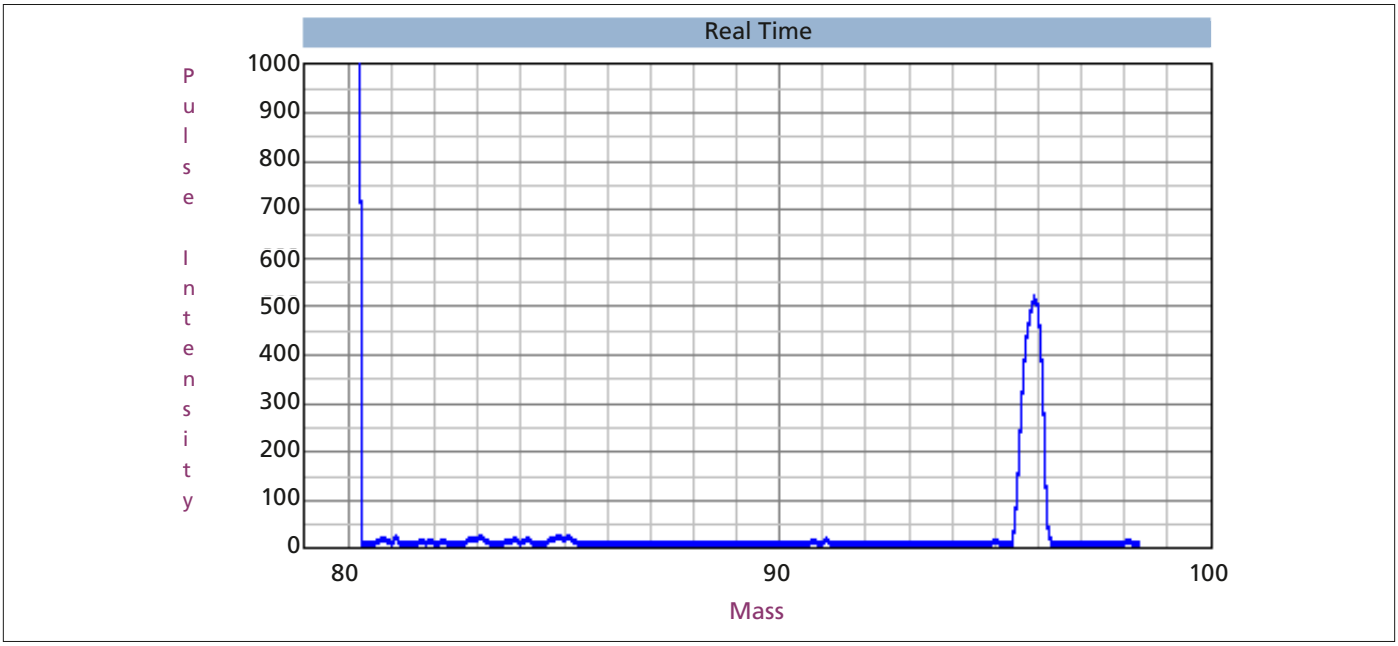

Figure 3. Product Ion scan of a 100-ppt mixed-element standard from mass 80 to 98 while Q1 transmits only ions at mass 80.

Another benefit of triple quadrupole technology is an observed improvement in abundance sensitivity, allowing an analyte residing on mass 1 amu lower than a high-concentration ion to be measured without interference. Due to the presence of two transmission quadrupoles, abundance sensitivity is greatly improved and both peaks are resolved down to the baseline. In blood, this technology eliminates the interference from high concentrations of Fe at masses 54 and 56 on Mn at mass 55.

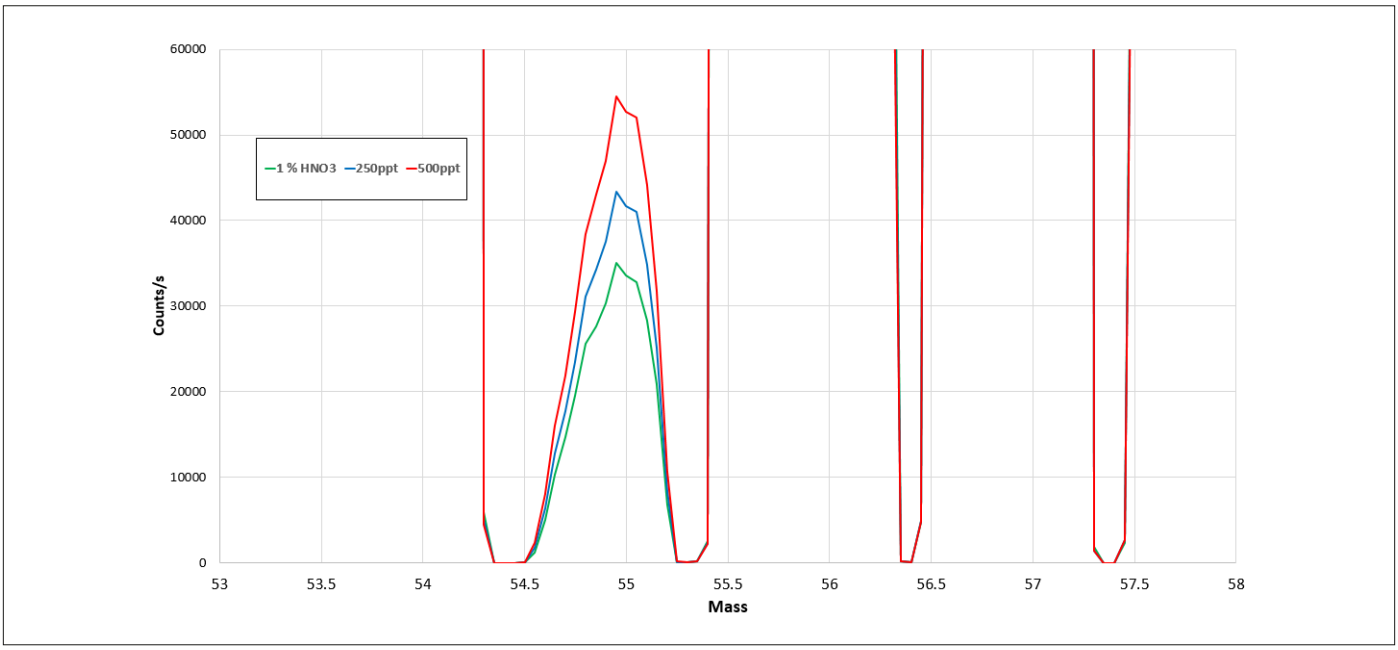

Figure 4. Great abundance sensitivity demonstrated by analyzing low levels of Mn (250 and 500 ppt) in the presence of high concentration of Fe (600 ppm using NH_3 as a reaction gas).

Since several elements are present at extremely low levels in non-exposed, normal-level samples, the first step was to establish the reporting limits (RLs) of the methodology, which were determined by multiplying the method detection limits (MDLs) by 5 (a common multiplier used in the industry). The MDLs were determined by analyzing the diluent 7 times and the standard deviations being multiplied by 50 (to account for the dilution factor) and by 3.14 to be within the 99% confidence limit. Table 2 shows both the MDLs and RLs in blood. At these levels, the most challenging aspect of the analysis is controlling contamination/background, which can arise from a variety of sources, including (but not limited to) sample handling, laboratory environment, and reagents.

To demonstrate the accuracy of the analysis, the reference samples (ClinChek, Levels I, II and III) were analyzed against calibration curves prepared from ClinCal by 50x, 100x and 200x dilution. The results appearing in Tables 3-5 demonstrate the accuracy of the methodology, with all recoveries being well within the certified values with the majority being within 15% or less from the median.

Table 2. Method Detection Limits (MDLs) and Reporting Limits (RLs) in Blood.

Element	Mode	Isotope Selection (Q1/Q3)	MDL (µg/L)	RL (µg/L)
Mg	Reaction Ammonia	24/24	1.429	7.147
P	Reaction Oxygen	31/47	7.163	35.813
Cr	Reaction Ammonia	52/52	0.020	0.099
Mn	Reaction Ammonia	55/55	0.021	0.105
Co	Reaction Ammonia	59/59	0.011	0.053
Ni	Reaction Ammonia	60/60	0.174	0.869
Cu	Reaction Ammonia	63/63	0.103	0.514
Zn	Reaction Ammonia	66/66	0.635	3.176
As	Reaction Oxygen	75/91	0.149	0.747
Se	Reaction Oxygen	80/96	0.332	1.661
Mo	Reaction Ammonia	98/98	0.013	0.065
Pd	Standard	106/106	0.024	0.118
Ag	Standard	107/107	0.010	0.052
Cd	Reaction Oxygen	111/111	0.023	0.114
Pt	Standard	195/195	0.026	0.130
Hg	Reaction Oxygen	202/202	0.074	0.369
Tl	Standard	205/205	0.007	0.037
Pb	Standard	208/208	0.040	0.199

Table 3. Results of ClinChek I and Control Range.

Element	Mode	Isotope Selection (Q1/Q3)	ClinChek I		Units
			Range	Results	
Mg	Reaction Ammonia	24/24	23.9-29.2	25.0	mg/L
P	Reaction Oxygen	31/47	312-467	348	mg/L
Cr	Reaction Ammonia	52/52	1.03-1.72	1.17	µg/L
Mn	Reaction Ammonia	55/55	7.09-10.60	7.59	µg/L
Co	Reaction Ammonia	59/59	1.24-1.87	1.46	µg/L
Ni	Reaction Ammonia	60/60	1.41-2.35	1.72	µg/L
Cu	Reaction Ammonia	63/63	0.542-0.813	0.65	mg/L
Zn	Reaction Ammonia	66/66	3.67-5.50	4.40	mg/L
As	Reaction Oxygen	75/91	4.34-6.51	5.12	µg/L
Se	Reaction Oxygen	80/96	60.2-90.3	75.3	µg/L
Mo	Reaction Ammonia	98/98	1.65-2.48	2.02	µg/L
Pd	Standard	106/106	0.578-0.867	0.71	µg/L
Ag	Standard	107/107	1.48-2.22	1.74	µg/L
Cd	Reaction Oxygen	111/111	0.987-1.48	1.24	µg/L
Pt	Standard	195/195	1.34-2.00	1.67	µg/L
Hg	Reaction Oxygen	202/202	0.885-1.640	1.30	µg/L
Tl	Standard	205/205	0.656-0.984	0.82	µg/L
Pb	Standard	208/208	43.6-65.3	54.5	µg/L

Table 4. Results of ClinChek II and Control Range.

Element	Mode	Isotope Selection (Q1/Q3)	ClinChek II		Units
			Range	Results	
Mg	Reaction Ammonia	24/24	31.4-38.5	33.7	mg/L
P	Reaction Oxygen	31/47	294-441	359	mg/L
Cr	Reaction Ammonia	52/52	4.20-7.01	5.35	µg/L
Mn	Reaction Ammonia	55/55	12.3-18.5	14.5	µg/L
Co	Reaction Ammonia	59/59	5.70-8.55	6.88	µg/L
Ni	Reaction Ammonia	60/60	3.74-5.61	4.17	µg/L
Cu	Reaction Ammonia	63/63	0.885-1.33	1.11	mg/L
Zn	Reaction Ammonia	66/66	5.02-7.53	6.12	mg/L
As	Reaction Oxygen	75/91	7.97-12.0	9.96	µg/L
Se	Reaction Oxygen	80/96	100-150	128	µg/L
Mo	Reaction Ammonia	98/98	3.58-5.37	4.43	µg/L
Pd	Standard	106/106	1.34-2.01	1.62	µg/L
Ag	Standard	107/107	3.50-5.25	4.22	µg/L
Cd	Reaction Oxygen	111/111	2.30-3.45	2.90	µg/L
Pt	Standard	195/195	1.95-2.92	2.44	µg/L
Hg	Reaction Oxygen	202/202	5.15-8.59	6.86	µg/L
Tl	Standard	205/205	3.35-5.03	4.02	µg/L
Pb	Standard	208/208	176-263	212	µg/L

Table 5. Results of ClinChek III and Control Range.

Element	Mode	Isotope Selection (Q1/Q3)	ClinChek III		Units
			Range	Results	
Mg	Reaction Ammonia	24/24	38.9-47.6	42.2	mg/L
P	Reaction Oxygen	31/47	306-459	365	mg/L
Cr	Reaction Ammonia	52/52	8.44-12.7	10.0	µg/L
Mn	Reaction Ammonia	55/55	17.7-26.5	21.0	µg/L
Co	Reaction Ammonia	59/59	10.4-15.7	12.8	µg/L
Ni	Reaction Ammonia	60/60	10.3-15.5	12.2	µg/L
Cu	Reaction Ammonia	63/63	1.34-2.01	1.72	mg/L
Zn	Reaction Ammonia	66/66	6.39-9.58	7.67	mg/L
As	Reaction Oxygen	75/91	15.5-23.2	18.9	µg/L
Se	Reaction Oxygen	80/96	134-201	170	µg/L
Mo	Reaction Ammonia	98/98	6.97-10.5	8.18	µg/L
Pd	Standard	106/106	3.38-5.07	4.05	µg/L
Ag	Standard	107/107	6.81-10.2	8.55	µg/L
Cd	Reaction Oxygen	111/111	5.06-7.59	6.28	µg/L
Pt	Standard	195/195	3.97-5.95	4.96	µg/L
Hg	Reaction Oxygen	202/202	8.59-12.9	9.23	µg/L
Tl	Standard	205/205	6.70-10.1	7.69	µg/L
Pb	Standard	208/208	340-510	406	µg/L

Figure 5 shows normalized results to the mean values in ClinChek I, II and III. As shown in Tables 3-5, results for all levels of CRM are within the control range and also very close to the mean values.

With the accuracy established, the stability of the methodology was explored next by analyzing pooled-blood samples over 6.5 hours and monitoring a QC standard (pooled-blood sample spiked with 2 ppb of a mixed standard) every 10 samples. The resultant plot (Figure 6) clearly demonstrates the exceptional stability of the multi-mode method and the lack of long-term drift; a direct result of the design of the NexION 5000 system.[3]

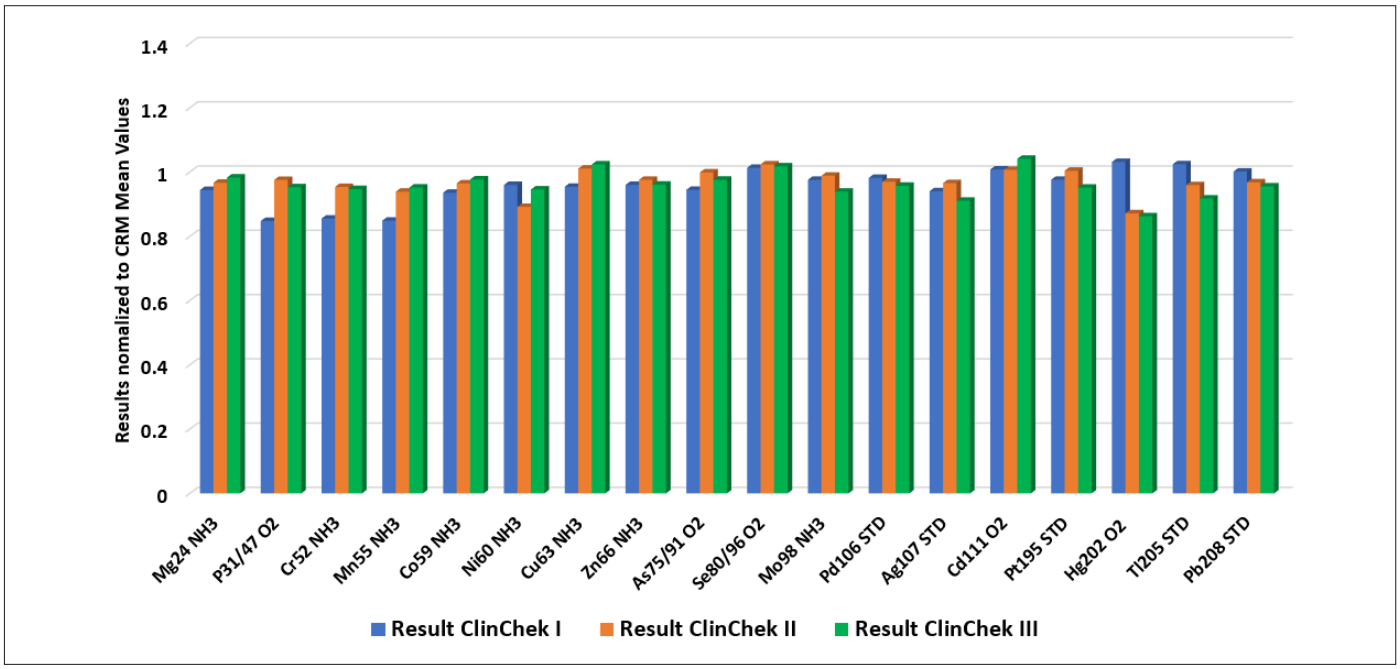

Figure 5. Analysis of ClinChek I, II and III Blood Reference Material. Results are normalized to ClinChek mean values.

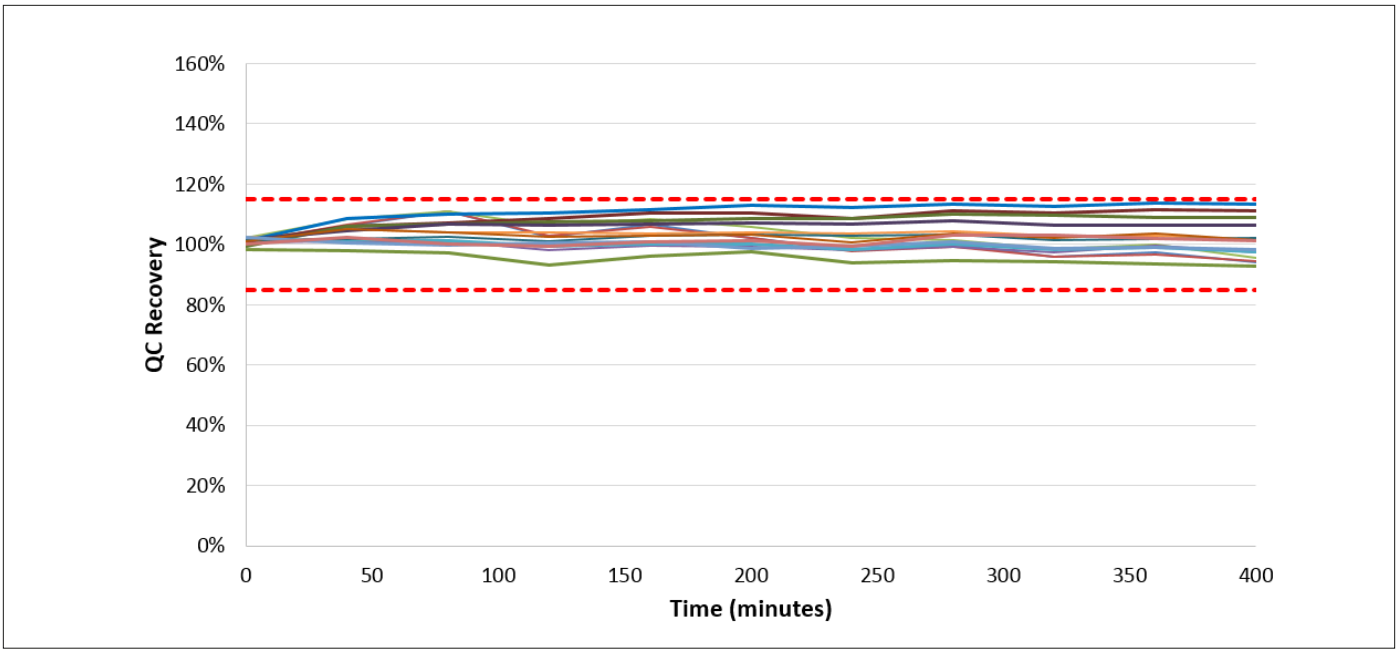

Figure 6. 6.5-h Stability run of QC standards in 50x diluted blood.

Conclusions

This work has demonstrated the ability of PerkinElmer's NexION 5000 multi-quadrupole ICP-MS to perform reproducible analyses of blood samples with outstanding stability over long sample run times. Accurate results were attained using a method with Standard MS/MS mode using NH_3 or O_2 as reaction gases and Mass Shift mode with O_2 as a reaction gas. Moreover, the Universal Cell and the ability to use up to four cell gases in a single method with triple quadrupole technology provides freedom to use the most appropriate interference-reduction strategies, guaranteeing superior, low-level analysis capabilities, limited only by external contamination.

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3. Badiei H., "Advantages of a Novel Interface Design for NexION 5000 ICP-MS" PerkinElmer Technical Note, 2020.

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