APPLICATION NOTE

ICP - Mass Spectrometry

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The NexION 2000: A Perfect Tool for the Determination of Trace Elements in Blood and Serum

Introduction

For many years, inductively coupled plasma mass spectrometry (ICP-MS) has been the technique of choice for trace element analysis in biological fluids. Researchers have found correlations between essential element levels and diseases, metabolic disorders, environmental exposures, and nutritional status.

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 ontains proteins, glucose, mineral salts, hormones, and red and white blood cells. Serum is derived from blood and has a similar composition, although it does not contain red or white cells or fibrinogens.

Since ICP-MS is a very effective technique for trace metal analysis due to its ability to see low levels in complex matrices, it is the ideal tool for the determination of trace metals in blood and serum, especially since normal levels for some analytes are extremely low. However, due to the complex nature of these matrices, matrix-derived spectral interference may be encountered during the ICP-MS analysis. This combination of low analyte concentrations, matrix-derived spectral interferences, and complex samples usually presents a challenge for ICP-MS analysis.

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PerkinElmer's NexION® 2000 ICP Mass Spectrometer is a perfect tool for the determination of trace elements in blood and serum due to a number of design characteristics. With its unique free-running solid-state RF generator, Triple Cone Interface, and Quadrupole Ion Deflector, the NexION 2000 has a high tolerance for total dissolved solids, allowing a simple "dilute and shoot" approach for sample preparation and analysis. The productivity configuration of the NexION 2000 is equipped with the FAST system that can greatly improve productivity in clinical testing laboratories. Finally, with the ability to use three cell gases in the Universal Cell, the NexION 2000 allows the most appropriate gas and mode to be used for analysis, providing the most effective removal of interferences and the lowest levels to be accurately measured, limited only by the cleanliness of the reagents and laboratory environment.

This work demonstrates the advantages of the NexION 2000 ICP-MS for trace elemental analysis in blood and serum and explores the ability to simplify sample preparation, which is a direct result of the NexION 2000's advantages.

Experimental

Samples and Sample Preparation

To determine the accuracy of the methodology, the following reference materials were used: Trace Elements in Blood and Trace Elements in Serum (UTAK® Laboratories Inc., Valencia, California, USA), ClinChek® Blood Control and ClinChek® Serum Control (Recipe Chemicals and Instruments GmbH, Munich, Germany). Two or three levels of each reference material (Normal, Elevated and High) were analyzed.

Blood and serum samples were prepared for analysis in two different ways: a 50x dilution with acidic or basic diluents. Since all standards and samples were prepared with the same amount of diluent, internal standards (Ga, Rh, and Ir) were added to the diluent.

The acidic diluent was a mixture of 0.5% HNO_3 (Veritas, Double Distilled, GFS Chemicals In., Powell, Ohio) + 0.05% Triton-X (Sigma-Aldrich™, St. Louis, Missouri, USA) + 2% methanol (Alfa Aesar™, Tewksbury, Massachusetts, USA), (all v/v) + 0.25 mg/L gold (Au) + internal standards. The nitric acid was required to keep elements stable in solution, but a low concentration was used to prevent precipitation of proteins and cell debris. The Triton-X was used to help rinse the spray chamber and to aid solubilization of proteins and cell membranes. Methanol was used to enhance both nebulization and ionization, while gold aided in mercury washout.

The basic diluent was a mixture of 0.5% TMAH (Sigma-Aldrich™) + 0.05% Triton-X (Sigma-Aldrich™) + 2% methanol (Alfa Aesar™), (all v/v) + 0.01% (w/v) APDC - ammonium pyrolidinedithio-carbamate (Sigma-Aldrich™) + internal standards. APDC, a strong complexing agent, is required to keep the metals in solution in the basic (TMAH) environment.

Calibration standards were prepared by diluting ready-to-use blood and serum standards with the acidic and the basic diluents. The matrix calibration standards consist of lyophilized blood and serum with the addition of commonly analyzed elements of known concentrations. The benefit of this approach is to eliminate the need to collect pooled blood or serum samples to perform matrix matched calibrations.

Analyses were done using acidic and basic sample preparations, and the results compared to determine which approach gives the most accurate results.

Instrumental Conditions

All analyses were carried out on a NexION 2000 ICP-MS using the SMARTintro™ High Throughput/High Matrix sample introduction system following the conditions and parameters shown in Table 1. To increase sample throughput, a high-productivity valve with a 1 mL sample loop was used in flow-switching mode. The carrier and rinse solutions were the same as the diluent, but without the addition of internal standards.

Table 1. Instrumental Parameters.

Parameter	Description/Value
Sample Introduction Rate	≈ 300 µL/min
Nebulizer	MEINHARD® Type C
Spray Chamber	Glass cyclonic
Spray Chamber Temperature	2 °C
RF Power	1600 W

For optimal performance, three-mode methods were used. Table 2 shows the elements, mass, and mode used for analysis in blood and serum. The ability to run three different cell gases (with or without Standard mode) in the same method from a single optimization file is unique to the NexION 2000 system. This approach results in enhanced ease-of-use and better performance. All required analytes can be measured at the same time without the need to divide them in separate panels.

Table 2. Elements and Analysis Mode.

Sample Type	Element	Mass	Analysis Mode	Cell Gas
Blood	Cr	52	Reaction	NH_3
	Mn	55	Reaction	NH_3
	Se	78	Reaction	O_2
	AsO	91	Reaction	O_2
	Cd	114	Reaction	O_2
	Hg	202	Reaction	O_2
	Tl	205	Standard	---
	Pb	208	Standard	---
Serum	Cr	52	Reaction	NH_3
	Cu	63	Collision	He
	Zn	66	Collision	He
	Se	78	Reaction	O_2
	Cd	114	Reaction	O_2
	Hg	202	Reaction	O_2

Results and Discussion

Since there are no specific requirements for blood or serum, the elements measured generally vary between laboratories: sometimes specific panels of elements are analyzed, while other analyses 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 analyzed in blood and serum (shown in Table 2).

The use of the Universal Cell along with the standard three cell gas channels on the NexION 2000 ICP-MS allowed the optimum conditions to be chosen for analysis, whether Controlled Reaction, Collision, or Standard mode. In Controlled Reaction mode, ammonia is the most effective cell gas for eliminating the carbon- and chloride-based interferences on chromium and manganese, permitting the user to obtain single-ppt detection limits for these elements in this matrix. On the other hand, oxygen is the most efficient reaction gas for eliminating the metal-oxide interferences on cadmium (MoO^+) and mercury (WO^+), while also removing a variety of interferences at m/z 78 for the analysis of selenium. Because of the multitude of interferences which can form on transition metals, helium in Collision mode is the best choice for interference removal. For heavy metals with no polyatomic interferences in urine, Standard mode is preferred.

Since several elements are present at extremely low levels in non-exposed, normal-level samples, the first step was to establish the reporting limits (RL) of the methodology, which were determined by multiplying the method detection limits (MDLs) by five (a common multiplier used in the industry). The MDLs were determined by analyzing the diluent seven times, with the standard deviation being multiplied by 50 (to account for the dilution factor) and 3.14 to be within the 99% confidence limit[1]. Table 3 shows both the MDLs and reporting limits in blood and serum. 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) reagents, laboratory environment, and sample handling.

To demonstrate the accuracy of the analysis, the reference materials were analyzed against calibration curves prepared in the calibration matrix. The results appear in Tables 4-7 and demonstrate the accuracy of the methodology, with all recoveries within 20% of the certified values, with the majority being within 10% or less.

Table 3. Detection Limits and Reporting Limits in Blood and Serum.

		Basic Diluent		Acidic Diluent	
Matrix	Element	MDL (µg/L)	RL (µg/L)	MDL (µg/L)	RL (µg/L)
Blood	Cr	0.068	0.340	0.053	0.265
	Mn	0.038	0.190	0.028	0.140
	Se	0.418	2.09	0.382	1.91
	AsO	0.153	0.765	0.131	0.655
	Cd	0.068	0.340	0.051	0.255
	Hg	0.065	0.195	0.055	0.275
	Tl	0.015	0.075	0.014	0.070
	Pb	0.066	0.330	0.045	0.225
Serum	Cr	0.130	0.650	0.054	0.270
	Cu	0.292	1.46	0.155	1.91
	Zn	0.943	4.72	2.22	11.1
	Se	0.665	3.32	0.872	4.36
	Cd	0.029	0.145	0.068	0.340
	Hg	0.163	0.815	0.136	0.680

Table 4. Analysis of ClinChek® Blood Reference Material (three levels).

Level 1		Basic Diluent		Acidic Diluent	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	1.25	0.98	79%	1.00	80%
Mn	8.01	7.59	95%	7.50	94%
Se	76.7	73.1	95%	74.77	98%
As	5.25	5.24	100%	5.48	104%
Cd	1.19	1.03	87%	1.22	103%
Hg	1.44	1.27	88%	2.03	141%
Tl	0.84	0.87	104%	0.86	102%
Pb	59.1	57.5	97%	57.0	96%
Level 2		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	5.49	5.03	92%	4.77	87%
Mn	14.9	14.3	96%	13.7	92%
Se	126	120	95%	115	91%
As	10.1	9.74	97%	10.0	99%
Cd	2.93	2.88	98%	2.85	97%
Hg	6.47	7.23	112%	7.10	110%
Tl	4.24	4.11	97%	4.22	100%
Pb	228	221	97%	219	96%
Level 3		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	10.9	11.1	102%	9.41	86%
Mn	21.4	20.8	97%	21.2	99%
Se	169	165	98%	167	98%
As	19.4	19.0	98%	19.1	98%
Cd	6.40	6.24	97%	6.41	100%
Hg	12.1	13.6	112%	14.1	116%
Tl	8.55	9.32	109%	8.98	105%
Pb	446	445	100%	457	103%

Table 5. Analysis of UTAK® Blood Reference Material (three levels).

Level 1		Basic Diluent		Acidic Diluent	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	0.75	0.65	90%	0.77	106%
Mn	10.7	8.51	80%	8.57	80%
Se	118	112	95%	121	102%
As	13.8	12.3	89%	12.8	92%
Cd	0.58	0.49	85%	0.55	94%
Hg	6.47	6.59	102%	7.09	110%
Tl	3.68	3.38	92%	3.68	100%
Pb	22.2	23.5	106%	22.1	100%
Level 2		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	15.2	14.0	92%	14.2	93%
Mn	31.4	27.5	88%	27.7	88%
Se	210	199	95%	205	98%
As	29.2	24.8	85%	25.3	87%
Cd	5.10	4.79	94%	4.81	94%
Hg	14.9	14.2	96%	15.7	106%
Tl	12.2	11.2	92%	12.4	101%
Pb	407	405	100%	410	101%
Level 3		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	58.7	56.8	97%	53.5	91%
Mn	92.0	84.9	93%	86.0	93%
Se	579	556	96%	550	95%
As	90.3	80.1	92%	13.7	94%
Cd	14.5	13.4	92%	13.7	94%
Hg	51.1	51.8	101%	52.5	103%
Tl	47.8	44.7	94%	48.0	101%
Pb	604	605	100%	605	100%

Table 6. Analysis of ClinChek® Serum Reference Material (two levels).

Level 1		Basic Diluent		Acidic Diluent	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	3.88	3.99	103%	3.89	100%
Cu	801	786	98%	803	100%
Zn	1320	1284	97%	1335	101%
Se	66.1	65.9	100%	66.9	101%
Cd	2.28	2.19	96%	2.20	96%
Hg	1.54	1.80	117%	1.59	103%
Level 2		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	7.49	7.83	105%	7.38	99%
Cu	1340	1245	93%	1360	102%
Zn	2040	1859	91%	2071	102%
Se	105	95.9	91%	98.8	94%
Cd	4.54	4.16	92%	4.65	102%
Hg	9.60	8.96	93%	10.1	105%

Table 7. Analysis of UTAK® Serum Reference Material (two levels).

Level 1		Basic Diluent		Acidic Diluent	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	< RL	---	---	---	---
Cu	1080	1088	101%	1173	109%
Zn	660	658	100%	696	106%
Se	103	110	107%	109	106%
Cd	0.21	0.21	100%	0.21	100%
Hg	NC	---	---	---	---
Level 2		**Basic Diluent**		**Acidic Diluent**	
Element	Certified (µg/L)	Measured (µg/L)	Recovery	Measured (µg/L)	Recovery
Cr	2.11	2.30	109%	2.28	108%
Cu	3060	3005	98%	3108	102%
Zn	2600	2480	95%	2566	99%
Se	282	302	107%	289	102%
Cd	9.30	8.92	96%	9.71	104%
Hg	---	---	---	---	---

RL = Reporting Limit

NC = Not Certified

In Tables 4-7, analysis data using acidic and basic diluents are reported. The plot in Figure 1 compares the % recoveries using of both approaches in the ClinChek® serum reference standards. These results indicate that the recoveries from both sample preparation methods are very similar, demonstrating the flexibility of using either strategy.

With the accuracy established, the stability of the methodology was explored next by analyzing pooled spiked blood samples (prepared in the acidic diluent) for 7 hours and monitoring a QC standard every ten samples. The resultant plot is shown in Figure 2 and clearly demonstrates the exceptional stability of the multi-mode method and lack of long-term drift, a direct result of NexION 2000's instrument design considerations.

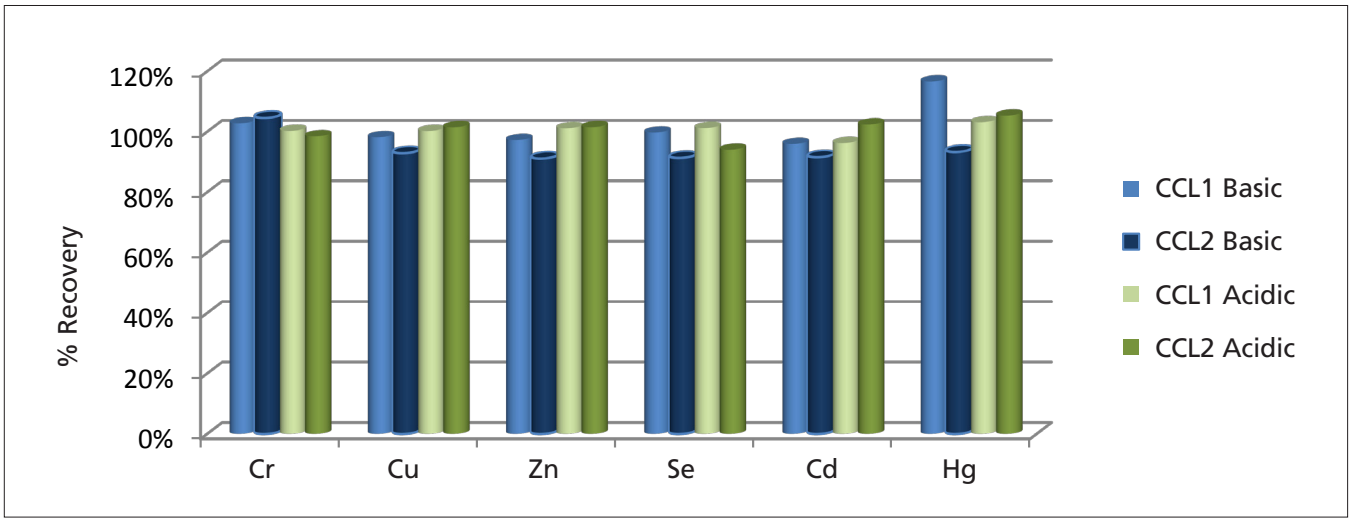

Figure 1. Recoveries in ClinChek® reference materials (Levels 1 and 2) with basic diluent (shades of blue) and acid diluent (shades of green).

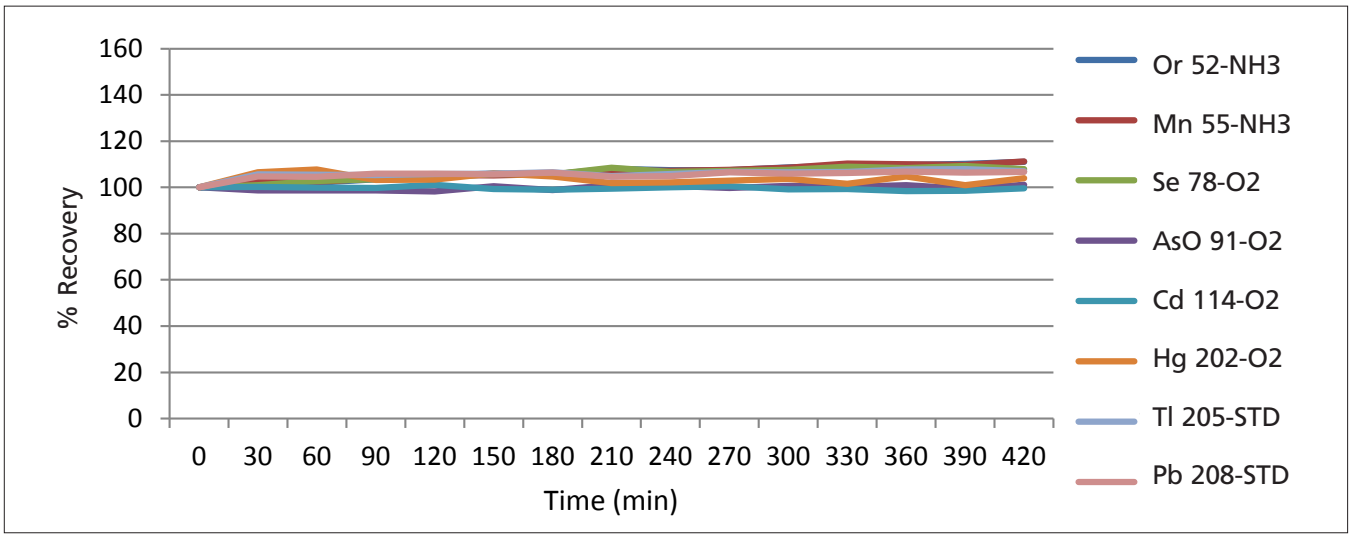

Figure 2. Stability of QC standard run every ten samples during a seven-hour run of blood samples.

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 2000 ICP-MS to perform accurate, stable analyses of blood and serum samples. Accurate results can be attained using either a basic or acidic diluent for standard and sample preparation. With the Universal Cell and the ability to use three cell gases in a single method, the most appropriate interference-reduction strategies can be used for interference elimination, guaranteeing superior low-level analysis capabilities, limited only by contamination.

References

1. Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11", 40 CFR, Part 136, Appendix B, Federal Register, 2016.

Consumables Used

Component	Description	Part Number
Carrier Tubing-1	Orange/white (0.64 mm id), flared, PVC, package of 12	N8142501
Carrier Tubing-2	Orange/red (0.19 mm id), flared, PVC, package of 12	N8145195
Drain Tubing	Gray/gray Santoprene (1.30 mm id), package of 12	N8145160

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