

## ICP - Mass Spectrometry

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## The Analysis of Urine for Trace Elements using the NexION 2000 ICP-MS

### Introduction

The determination of trace elements in biological fluids presents a challenge for a number of reasons, including the complex nature of the matrices and the widely

varying concentrations of the metals which are present. Among the most commonly analyzed biological fluids (urine, blood, serum), urine is unique in that its main function is waste removal from biological systems. As a result, urine is composed of a complex mixture of components which can vary widely between samples and can be affected by food, environmental, and industrial exposures. For these reasons, both matrix and analyte levels of urine often vary significantly between samples, posing a challenge for analysis.

There are two main challenges that must be overcome when measuring trace metals in urine: the presence of matrix-induced polyatomic interferences and the very low levels of some elements used to assess contaminant exposure, such as arsenic (As), cadmium (Cd), and mercury (Hg), among others. With the ability to use three cell gases in the Universal Cell, PerkinElmer's NexION® 2000 ICP Mass Spectrometer (ICP-MS) 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.

Owing to the complexity of the urine matrix, calibration is usually done using a matrix-matching approach. This work demonstrates the capabilities of the NexION 2000 ICP-MS to accurately measure commonly-analyzed elements at both normal and elevated levels using an aqueous non-matrix-matched calibration. This capability is mainly due to a new solid-state free running RF generator that offers improved plasma coupling and exceptional long-term stability. Adding to the RF generator, the NexION 2000's three gas channel flexibility offers the ability to target various interferences in order to get the best method detection limits (MDLs).

## Experimental

### Samples and Sample Preparation

To determine the accuracy of the methodology, three sets of reference materials were used: Trace Elements in Urine (UTAK® Laboratories Inc., Valencia, California, USA), ClinChek® Urine Control see Consumables Used table for part number, and NIST 2670a Toxic Elements in Freeze-Dried Urine (National Institute of Standards and Technology, Rockville, Maryland, USA). Two levels of each reference material (Normal Range/Level 1, High Range/Level 2) were analyzed.

Urine samples were prepared for analysis by 10x dilution with a mixture 0.5% HNO<sub>3</sub> (Veritas, Double Distilled, GFS Chemicals Inc., 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 is required to keep elements stable in solution, but a low concentration is used to prevent precipitation of proteins. The Triton-X is used to lyse any potential cells that could have found their way to the urine samples. Methanol was used to enhance both nebulization and ionization, while gold aids in mercury washout. Finally, because all standards and samples are prepared with the same amount of diluent, the most accurate way to add internal standards is to add them to the diluent.

In order to establish the suitability of the non-matrix matched approach, calibration standards were prepared in two ways: (1) in a lyophilized urine solution and (2) in the diluent, without any matrix matching. Analyses were done against both calibration curves, and the results compared.

### Instrumental Conditions

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

For optimal performance, a four-mode method was used; Table 2 shows the elements, mass, and mode use for analysis. The ability to run three different cell gases (along with Standard mode) in the same method means no compromises, resulting in enhanced accuracy and MDLs while maintaining ease-of-use.

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

Table 2. Elements and Analysis Mode.

Element	Mass	Analysis Mode	Cell Gas
Cr	52	Reaction	Ammonia
Mn	55	Reaction	Ammonia
Co	59	Collision	Helium
Ni	60	Collision	Helium
Cu	63	Collision	Helium
AsO	91	Reaction	Oxygen
Se	78	Reaction	Oxygen
Mo	95	Collision	Helium
Cd	111	Reaction	Oxygen
Sb	121	Standard	---
Hg	202	Reaction	Oxygen
Tl	205	Standard	---
Pb	208	Standard	---

## Results and Discussion

As a result of the lack of regulatory requirements for trace elements in urine, the elements measured generally vary by lab: some analyze individual elements and others multi-element panels. Hence, the elements chosen for this work represent a typical cross-section of those analyzed by a variety of labs.

The use of the Universal Cell along with the standard three cell gas channels on the NexION 2000 system 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<sup>+</sup>) and mercury (WO<sup>+</sup>), 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 samples, the first step was to establish the reporting limits (RL) of the methodology, which were determined by multiplying the 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 10 (to account for the dilution factor) and 3.14 to be within the 99% confidence limit<sup>1</sup>. Table 3 shows both the MDLs and reporting limits. 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, all three reference materials were analyzed against calibration curves prepared in the matrix-matched calibration. The results appear in Tables 4-6 and show the accuracy of the methodology, with all recoveries within 20% of the certified values, with the majority being within 10%.

Table 3. Detection Limits and Reporting Limits in Urine

Element	MDL (µg/L)	Reporting Limits (MDL*5) (µg/L)
Cr	0.007	0.037
Mn	0.005	0.026
Co	0.006	0.029
Ni	0.061	0.305
Cu	0.034	0.171
As	0.021	0.104
Se	0.029	0.104
Mo	0.016	0.079
Cd	0.010	0.050
Hg	0.039	0.144
Sb	0.004	0.021
Tl	0.004	0.020
Pb	0.008	0.042

Table 4. Analysis of ClinChek® Urine Reference Material.

Element	ClinChek® Level 1			ClinChek® Level 2		
	Certified (µg/L)	Experimental (µg/L)	% Recovery	Certified (µg/L)	Experimental (µg/L)	% Recovery
Cr	4.07	3.97	98	19.9	20.0	100
Mn	3.91	3.88	99	19.4	19.4	100
Co	2.03	1.94	96	34.7	35.3	102
Ni	5.92	5.94	100	43.0	42.4	99
Cu	36.7	36.7	100	91.9	95.6	104
As	43.0	42.3	98	83.3	85.8	103
Se	29.9	28.6	96	83.3	86.0	103
Mo	23.9	23.0	96	101	102	101
Cd	2.46	2.37	96	14.4	14.5	101
Hg	2.30	2.62	114	17.3	17.4	101
Sb	11.1	11.2	101	45.4	45.3	100
Tl	7.24	7.12	98	19.0	18.9	99
Pb	24.0	22.5	94	65.0	64.8	100

Table 5. Analysis of UTAK® Urine Reference Materials.

Element	Normal Levels			High Levels		
	Certified (µg/L)	Experimental (µg/L)	% Recovery	Certified (µg/L)	Experimental (µg/L)	% Recovery
Cr	2.94	2.81	95	9.42	9.17	97
Mn	1.19	1.19	100	2.21	2.50	113
Co	1.05	1.03	98	7.50	7.64	102
Ni	4.14	3.62	87	35.7	34.6	97
Cu	123	118	96	165	161	98
As	9.43	8.84	94	107	108	101
Se	55.2	64.2	116	78.0	87.2	112
Mo	77.8	81.1	104	102	106	104
Cd	0.02	< RL	---	4.62	4.27	92
Hg	0.08	< RL	---	49.2	51.1	104
Sb	NC	---	---	NC	---	---
Tl	NC	---	---	NC	---	---
Pb	0.26	0.28	107	126	123	98

RL = Reporting Limit

NC = Not Certified

Table 6. Analysis of NIST 2670a Urine Reference Material.

Element	Low Level			High Level		
	Certified (µg/L)	Experimental (µg/L)	% Recovery	Certified (µg/L)	Experimental (µg/L)	% Recovery
Cr	NC	---	---	NC	---	---
Mn	NC	---	---	99.0	105	106
Co	0.166	0.146	88	51.2	53.5	105
Ni	NC	---	---	100	103	103
Cu	NC	---	---	110	99	90
As	NC	---	---	220	225	102
Se	NC	---	---	230	274	120
Mo	NC	---	---	114	121	106
Cd	0.059	RL	---	5.16	5.26	102
Hg	0.066	< RL	---	95.1	96.3	101
Sb	0.971	0.982	101	0.824	0.784	95
Tl	0.016	< RL	---	5.42	5.54	102
Pb	0.490	0.478	97	250	250	100

RL = Reporting Limit  
 NC = Not Certified

To demonstrate that the robust plasma of the NexION 2000 ICP-MS can overcome ionization effects normally seen with urine, the ClinChek® reference materials were also measured against a calibration curve prepared in the urine diluent. The plot in Figure 1 compares the results of both ClinChek® samples measured against both acid and urine calibration curves. These results indicate that although the urine calibrations yield recoveries closer to 100%, the recoveries from both calibration curves are within 10% of the certified values, thus demonstrating the ability of the NexION 2000 system to run urine samples without the need to matrix match standards, yielding considerable savings on laboratory operating cost.

With the accuracy established, the stability of the methodology was explored next by analyzing urine samples over 10 hours and monitoring a calibration check standard as a QC every 10 samples. Figure 2

shows a plot of all elements in the check standard, demonstrating the exceptional stability of the multi-mode methodology and lack of long-term matrix effects, with a variation of ± 5% over 10 hours.

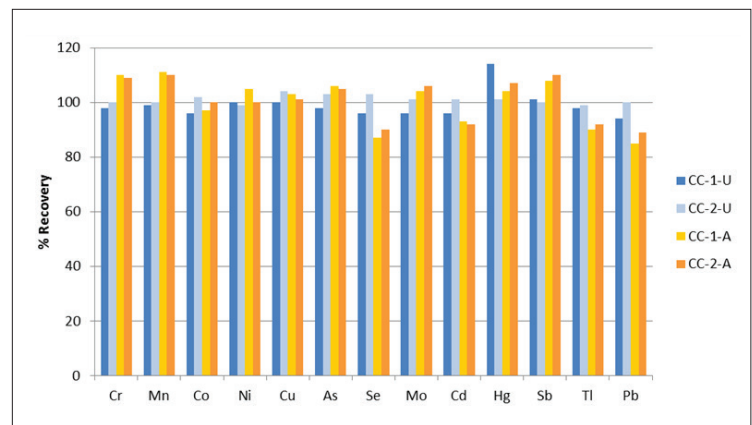


Figure 1. Recoveries in ClinChek® reference materials (Levels 1 and 2) against both urine calibrations (shades of blue) and acid calibrations (shades of orange).

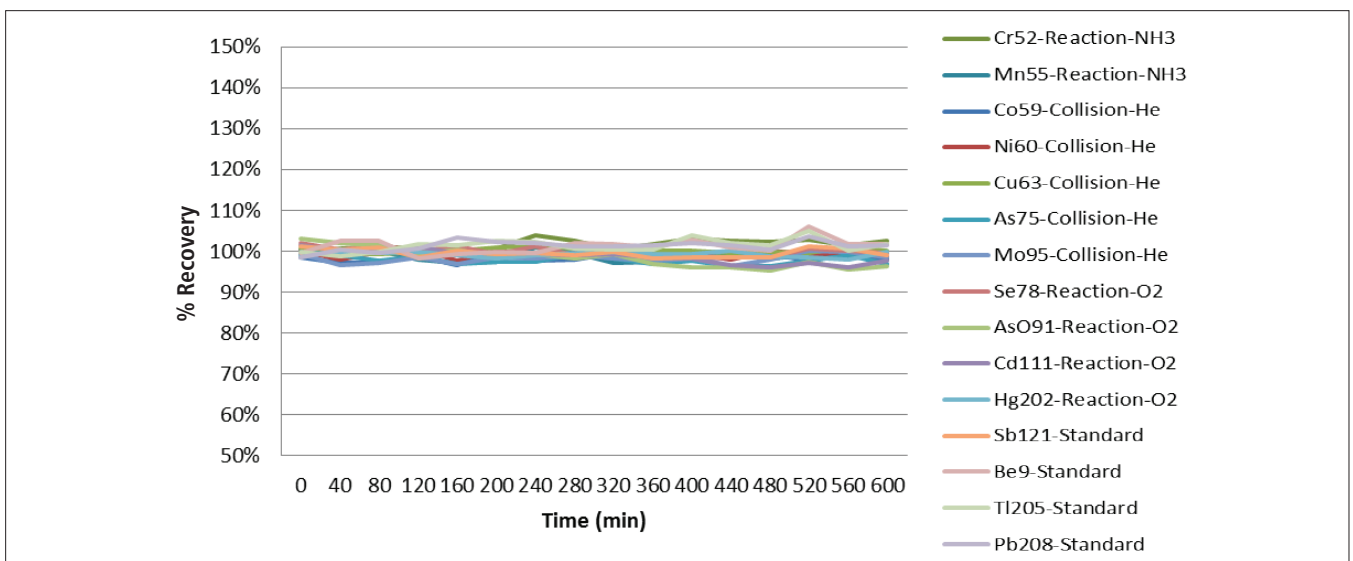


Figure 2. Stability of QC check standard run every ten samples during a 10-hour run of urine samples.

## Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 2000 ICP-MS to perform accurate, stable analyses of urine samples. The unique, new solid-state RF generator produces a robust plasma allowing accurate results to be attained using either matrix-matched or acid-based calibration curves. With the Universal Cell and the ability to use three cell gases in a single method, the most appropriate interference-reduction strategies and gases can be used for interference elimination, guaranteeing superior low-level analysis capabilities, limited only by contamination. The use of three gas channels in a single run is essential for obtaining the best interference removal without sacrificing on sensitivity and thereby resulting in improved accuracy and MDLs.

## 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	N8145201
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
Instrument Calibration Standard 2	100 mg/L Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn – 125 mL	N9301721
Mercury Standard	10 mg/L Hg – 125 mL	N9300253
Gold Standard	1000 mg/L – 125 mL	N9303759
Internal Standard Mix	Sc = 200 mg/L; Ga = 20 mg/L; Rh, In, Ir, Tm = 10 mg/L – 125 mL	N9307738
Autosampler Tubes	Metal-free, racked, white caps, 500 per package	N0776118 (15 mL) N0776116 (50 mL)
ClinCal® Urine Calibrator	5x10 mL vials of lyophilized urine	N8145490
ClinChek® Urine Control	Levels 1 and 2, 5x10 mL vials of each	N8145493