APPLICATION NOTE

ICP - Mass Spectrometry

Authors:

David Bass

Daniel Jones

PerkinElmer, Inc.
Shelton, CT USA

Determination of Trace Metals in Human Urine Using the NexION 300/350 ICP-MS

Introduction

The monitoring of trace metals in human urine plays an important role in research. Traditionally, urine analysis has been accomplished by graphite furnace atomic absorption (GFAA). However, when large numbers of samples are analyzed for multiple elements, GFAA becomes very cumbersome and restrictive, since it can only determine one element at a time. Additionally, the detection capability of ICP-MS for many elements is far superior to GFAA.[1] The benefits of ICP-MS are well-recognized and include:

- Superior detection-limit capability[2]
- Enhanced sensitivity
- Higher sample throughput
- Well-defined interferences[3]
- Reliable isotopic analysis
- Detection of elemental species using HPLC[4]

However, human urine is a complex matrix containing high levels of urea, uric acid, proteins, fats, sodium, potassium, bicarbonate and chloride, as represented in Figure 1, which shows chemical breakdown of the approximately 1.4 liters of urine passed by a typical adult on a daily basis. These components can cause signal suppression during ICP-MS analysis. In addition, there is the potential for signal drift caused by matrix deposition on the interface cones and ion-lens system. Another potential problem is the formation of polyatomic interferences caused by the combination of matrix components with aqueous and plasma species.

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Experimental

Instrumentation

For this study, the PerkinElmer NexION® 300D, an innovative ICP-MS, was used to analyze a group of UTAK® freeze-dried urine SRM samples. This instrument is ideally suited for the analysis of high-matrix samples because of its unique design. For the first time, a single ICP-MS instrument offers both the simplicity and convenience of a traditional collision cell with kinetic energy discrimination (KED) and the superior interference-reduction capabilities and detection limits of the Dynamic Reaction Cell™ (DRC™). With this design, analysts can now choose the most appropriate collision/reaction cell technology for a specific application, without any restrictions to the type of gases that can be used.

The NexION 300 ICP-MS also features a unique triple cone interface. Unlike other systems which only have sampler and skimmer cones, this instrument also includes a hyper skimmer cone to tightly define and focus the ion beam. Pressure within the interface is reduced in smaller steps, providing less dispersion of ions and preventing sample deposition on internal surfaces. All three cones can be quickly and easily removed, cleaned or replaced – an important point for the analysis of urine, which contains high levels of salts and organic materials.

The ion beam emerges from the triple cone interface and enters a quadrupole ion deflector (QID) which is designed around a proprietary, miniaturized quadrupole. The QID bends the ion beam 90 degrees, focusing ions of a specified mass into the cell. Neutrals, non-ionized species, and photons are not affected by the voltages and pass through directly to vent, never impacting any of the surfaces within the QID. Therefore, the voltages within the QID remain constant, resulting in low backgrounds, minimal drift, and exceptional stability even when running the most challenging matrices.

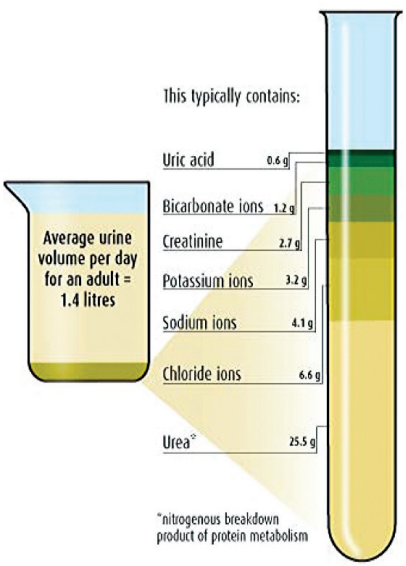

Figure 1. Chemical breakdown of the 1.4 liters of urine passed by a typical adult on a daily basis.

Sample Preparation

Two UTAK® (Valencia, CA) freeze-dried urine standard reference materials (SRMs) were chosen for this study: normal- and high-range urines (UTAK®-12111, Lot # 3500; UTAK®-12110, Lot # 3499). Before analysis, these control samples are reconstituted with 5.0 mL of 1% hydrochloric acid as per the enclosed certificate instructions, then diluted 10-fold with deionized water and preserved with 1% nitric acid. Both acids were Optima® grade (Fisher Scientific®).

To minimize matrix effects during ionization, calibration standards (0.1, 1, 5, and 10 µg/L) were prepared in a pooled urine sample.

Methodology

Urine, like other biological materials, contains high levels of carbonaceous materials, chlorides and other dissolved solids which can cause both spectral and matrix-induced interferences on the analytes of interest. Therefore, accurate trace-metal determinations in this matrix can be difficult. For example, chloride and carbon ions form the polyatomic species ArC^+, $ArCl^+$, ArN^+ and ClO^+, which interfere with the determination of Cr^+, As^+, Mn^+ and V^+. Therefore, it is important to reduce the impact of these interferences by using cell technology.

Although both Reaction and Collision/KED modes are available, the analysis was performed using Reaction mode because of its superior detection capability through the use of ion-molecule reaction chemistries. It was felt that the extremely low quantitation levels, especially with the 10-fold dilution of the normal-range UTAK® SRM, necessitated the use of DRC technology. With that in mind, ammonia (NH_3) was used for the measurement of several of the transition elements, while oxgen (O_2) was used for the determination of arsenic.

Ammonia is universally recognized as the best reaction gas to reduce argon-based spectral interferences. The reason for this is that the reactivity of NH_3 with argon ions is extremely rapid and exothermic, whereas its reaction rate with first-row transition metals is much slower. The reduction of $^{40}Ar^{12}C^+$ on $^{52}Cr^+$ with ammonia serves as an example of this concept. Since both of these species exist at mass 52, low levels of Cr cannot be measured in the presence of carbon. However, NH_3 reacts much more rapidly with ArC^+ ($K \approx 10^{-10}$) than with Cr^+ ($K \approx 10^{-12}$) through the following mechanism:

$$ArC^+ + NH_3 \longrightarrow Ar + C + NH_3^+ \qquad K \approx 10^{-10}$$

$$Cr^+ + NH_3 \longrightarrow Cr(NH_3)^+ \qquad K \approx 10^{-12}$$

The net result is an increase in signal-to-background through the elimination of ArC^+, thus allowing trace levels of Cr to be measured. This process is similar for the reduction of other polyatomic interferences using the Reaction mode.

The optimization plot for ^{52}Cr in the presence of high concentrations of carbon ions (isopropanol) is shown in Figure 2. The x-axis shows the NH_3 cell gas flow rate, while the y-axis represents the signal intensity. It is evident that the signal intensity of the $^{40}Ar^{12}C^+$ in the blank is significantly reduced, while the signal for the 1 ppb ^{52}Cr is largely unaffected. The initial apparent drop in the Cr signal from NH_3 = 0.1-0.3 mL/min is actually the reduction of ArC^+; 1 ppb Cr cannot be seen in the presence of such a high concentration of carbon at such low ammonia flows. At an NH_3 flow rate of approximately 0.7 mL/min, the ArC^+ interference has been reduced to less than 100 counts, which represents a reduction of 4-5 orders of magnitude from the original level. The dynamic bandpass tuning of the DRC technology immediately ejects NH_3^+ ions generated in the cell, thus avoiding undesirable side reactions taking place (Note: This optimized DRC bandpass tuning is represented by the RPq values shown in Table 2 – Page 4). As a result, only ^{52}Cr ions exit the cell and enter the analyzer quadrupole. Figure 3 shows the Cr calibration curve (0-5 µg/L Cr) in urine for $^{52}Cr^+$. The linearity of the curve at these levels provides evidence that the ArC^+ interference has been removed.

For the determination of arsenic, the analyst can leverage the DRC's ability to move arsenic to a new analytical mass, away from the interferences. In urine, the main interferences on $^{75}As^+$ are $^{40}Ar^{35}Cl^+$ and $^{40}Ca^{35}Cl^+$. Although $ArCl^+$ reacts rapidly with various cell gases, $CaCl^+$ is very unreactive due to the extremely high Ca-Cl bond strength. As a result, $CaCl^+$ cannot be eliminated through reaction chemistry. Although Collision mode would address both of these interferences, the loss of As sensitivity is great, which would make trace-level measurements difficult.

A better alternative would be to use oxygen as the cell gas and take advantage of the rapid reaction between As^+ and O_2 to form $^{75}As^{16}O^+$ at m/z 91, as shown previously.[5] The conversion of As^+ to AsO^+ is illustrated in Figure 4. In this figure, the X axis shows the gas flow, and the Y axis shows the intensity; the red curve is the $^{75}As^+$ signal and the blue curve is the $^{75}As^{16}O^+$ signal, both as a function of oxygen flow. This data clearly shows that as the As^+ signal decreases, the AsO^+ signal increases, demonstrating the conversion of As^+ to AsO^+.

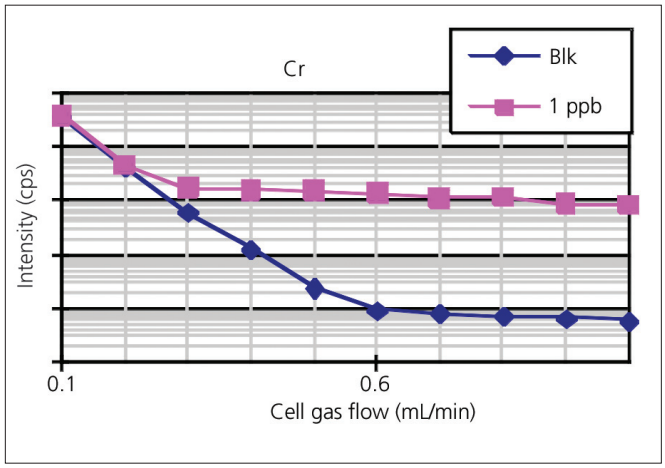

Figure 2. NH_3 Cell gas optimization of ^{52}Cr in the presence of $^{40}Ar^{12}C^+$ using reaction chemistry.

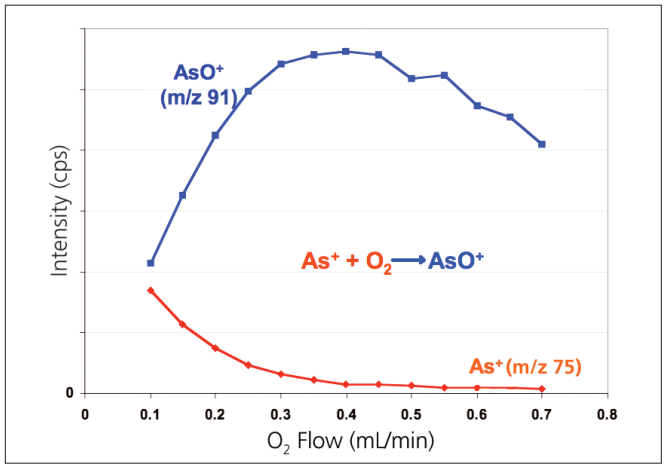

Figure 4. Optimization of the oxygen gas flow in the conversion of $^{75}As^+$ to $^{75}As^{16}O^+$.

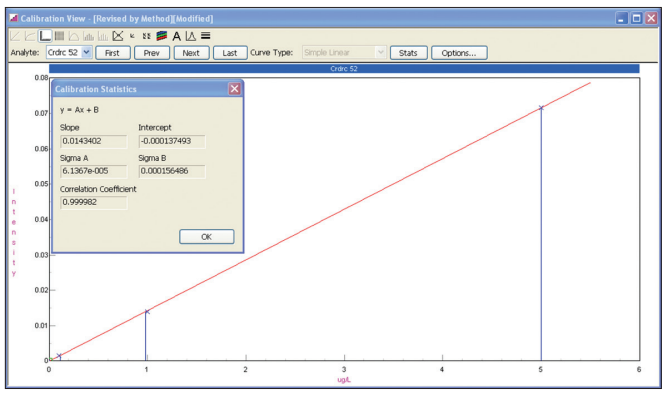

Figure 3. Calibration plot of 0.1, 1.0 and 5.0 µg/L of $^{52}Cr^+$ in urine.

Instrument Operating Parameters

Instrument operating conditions for the analysis of urine are shown in Table 1; reaction cell conditions appear in Table 2. A high RF power (1500 watts) is important to break down the urine and reduce the effects of matrix suppression. The combination of high RF power in conjunction with a low sample-uptake rate leads to a more energetic plasma, which promotes more complete ionization, reducing deposition on the sampler and skimmer cones, thereby minimizing signal drift.

The elements determined in Reaction mode (As, Cr, Co, Cu, Mn, V) are shown in Table 2; all other elements were determined in the Standard mode. Both sets of elements were combined into a single method. Changeover time between Standard and Reaction modes was approximately 10 seconds.

Table 1. Instrument conditions used for the analysis of UTAK® freeze-dried urine.

Parameter	Setting
Sample Introduction System	Baffled Cyclonic Spray Chamber with a Meinhard Low Flow nebulizer
Sample Uptake Rate	0.3 mL/min
Sampler and Skimmer Cones	Nickel
Forward Power	1500 watts
Nebulizer Gas Flow	0.8 L/min
Sweeps	20
Points per Peak	1
Replicates	3
Dwell Time	100 ms
Modes	Standard and Reaction
Time to Change Modes	10 s
Internal Standards	Indium (^{115}In) for all elements except Yttrium (^{89}Y) for ^{66}Zn

Table 2. Reaction gases and gas flows used with the cell RPq values for the determination of As, Cr, Co, Cu, Mn, V in UTAK® normal and high level freeze-dried urine SRMs, using Reaction mode.

Analyte (Mass)	Reaction Gas	Gas Flow (mL/min)	DRC Setting (RPq Value)
Arsenic Oxide (91)	Oxygen	0.7	0.65
Chromium (52)	Ammonia	0.7	0.75
Cobalt (59)	Ammonia	0.7	0.75
Copper (65)	Ammonia	0.7	0.75
Manganese (55)	Ammonia	0.7	0.75
Vanadium (51)	Ammonia	0.7	0.75

Results

Results for the UTAK® SRMs are shown in Tables 3 (normal level) and 4 (high level). The "Expected Range" is the lowest and the highest value obtained by these techniques. The "Reported Values" are typical results obtained in this study. All the reported values fall within the expected range, thus validating the method.

Table 3. Results for the normal-level UTAK® freeze-dried urine SRM.

Analyte (Mass)	Reported Value (µg/L)	Expected Range (µg/L)
*Arsenic as AsO (91)	9.2	8 to 11
*Chromium (52)	1.1	1.0 to 1.4
*Cobalt (59)	1.8	1.4 to 2.0
*Copper (65)	118	100 to 136
Lead (208)	0.56	0.5 to 0.7
*Manganese (55)	3.2	2.5 to 3.3
Molybdenum (98)	76	60 to 82
*Vanadium (51)	0.69	0.5 to 0.7
Zinc (66)	842	666 to 900

**denotes Reaction mode*

Table 4. Results for the high-level UTAK® freeze-dried urine SRM.

Analyte (Mass)	Reported Value (µg/L)	Expected Range (µg/L)
Aluminum (27)	35	32-44
*Arsenic as AsO (91)	99	88-116
Cadmium (114)	5.0	4.2-5.6
*Chromium (52)	7.6	6.3-8.5
*Copper (65)	171	143-193
Lead (208)	132	111-150
*Manganese (55)	3.9	3.0-4.0
Molybdenum (98)	98	75-101
*Vanadium (51)	10.8	9-12
Zinc (66)	1128	1112-1504

**denotes Reaction mode*

Conclusion

This work has shown that the innovative design of PerkinElmer's NexION 300 ICP-MS is ideally suited for trace-metal determination in urine in research applications. The combination of innovative instrumental-design considerations along with energetic plasma conditions and reaction cell technology allows for the accurate determination of both trace and elevated levels of elements in urine.

References

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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