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Development and validation of a biomonitoring method to measure As, Cr, and Ni in human urine samples by ICP-UCT-MS

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Abstract

We developed an inductively coupled plasma mass spectrometry (ICP-MS) method using Universal Cell Technology (UCT) with a PerkinElmer NexION ICP-MS, to measure arsenic (As), chromium (Cr), and nickel (Ni) in human urine samples. The advancements of the UCT allowed us to expand the calibration range to make the method applicable for both low concentrations of biomonitoring applications and high concentrations that may be observed from acute exposures and emergency response. Our method analyzes As and Ni in kinetic energy discrimination (KED) mode with helium (He) gas, and Cr in dynamic reaction cell (DRC) mode with ammonia (NH_3) gas. The combination of these elements is challenging because a carbon source, ethanol (EtOH), is required for normalization of As ionization in urine samples, which creates a spectral overlap ($^{40}Ar^{12}C^+$) on ^{52}Cr. This method additionally improved lab efficiency by combining elements from two of our previously published methods(Jarrett et al., 2007; Quarles et al., 2014) allowing us to measure Cr and Ni concentrations in urine samples collected as part of the National Health and Nutrition Examination Survey (NHANES) beginning with the 2017–2018 survey cycle. We present our rigorous validation of the method selectivity and accuracy using National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM), precision using in-house prepared quality control materials, and a discussion of the use of a modified UCT, a BioUCell, to address an ion transmission phenomenon we observed on the NexION 300 platform when using higher elemental concentrations and high cell gas pressures. The rugged method detection limits, calculated from measurements in more than 60 runs, for As, Cr, and Ni are 0.23 μg L-1, 0.19 μg L-1, and 0.31 μg L-1, respectively.

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Declaration of competing interest

There are no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113713.

Keywords

Arsenic (As); Chromium (Cr); Nickel (Ni); ICP-MS; Dynamic reaction cell (DRC); Kinetic energy discrimination (KED); Biomonitoring

1. Introduction

Arsenic (As), chromium (Cr), and nickel (Ni) are naturally occurring elements of public health interest due to the potential for human exposure from both natural and man-made sources.(2005, b) Arsenic compounds have been used as a wood preservative and in agriculture as pesticides or an animal feed additive. Human exposure to As has also occurred through intentional poisoning(Gensheimer et al., 2010) and accessibility of toxic, arsenic containing products necessitates public health concerns. Cr and Ni are used to make stainless steel and can also be found in metallic plating, tanned leather (Cr) and jewelry (Ni). No forms of As are considered essential for human biological functions, but exposure to the inorganic forms are generally the greatest threat to human health(U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 2012a). Cr(III) is non-toxic and essential to human biological functions, while Cr(VI) is a known carcinogen(U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 2012b). Nickel essentiality and toxicity to humans is less well studied. Nickel may be a necessary, essential trace element but some compounds are known to be carcinogens(U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 2005). Exposure to As, Cr, or Ni can affect nearly every major biological system ranging from respiratory and dermal irritation, to neurological, cardiovascular, gastrointestinal, and reproductive effects(U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 2005, 2012a, b).

The Centers for Disease Control and Prevention’s (CDC) National Biomonitoring Program develops and applies analytical methods to measure chemicals in human urine, blood, and serum samples as a way to assess exposure of populations to these chemicals(Wang et al., 2014). Biomonitoring surveys, such as the National Health and Nutrition Examination Survey (NHANES), are used to establish population reference ranges and track exposure trends over time. Biomonitoring data can show how regulatory public health efforts, such as lowering the drinking water limits for As, affect a population’s exposure to chemicals. These same methods are also used to identify acute exposures during response to chemical terrorism and other public health emergencies.

ICP-MS is well suited to measure trace and toxic elements in clinical samples for these applications because of its sensitivity, multi-element capability and wide dynamic range. The high-power plasma allows for a simple dilution sample preparation scheme instead of a time-consuming sample digestion. Also, modern ICP-MS instruments have improved the specificity of the technique by using technology such as collision and reaction cells and MS/MS technology to reduce or eliminate many mass spectral interferences. Our laboratory previously developed separate methods to test urine total As(Jarrett et al., 2007) and urine Cr

and Ni(Quarles et al., 2014). Here we report combining these two methods into a single method, to improve laboratory efficiency, and to expand the calibration range to make the method applicable for both low concentrations of biomonitoring applications and high concentrations that may be observed from acute exposures and emergency response situations.

The BioUCell is an instrument upgrade from PerkinElmer replacing the standard UCT on the NexION 300D to eliminate “cross-talk” or ion signal over-recovery in DRC mode. The cross-talk we observed occurs when the reaction cell is overly-populated with ions from a sample with a high total ion concentration. The ions in the reaction cell, all positively charged, repel each other and some are forced toward the cell exit and the instrument detector. This effect makes the measured ion count at the detector a factor of the concentration of an element in the sample, but also on the concentration of all other ions in the reaction cell. The BioUCell and the axial field voltage (AFV) correct cross-talk so that high concentration samples can be accurately measured.

Combining our two methods into one presented challenges in addressing non-spectral and spectral interferences. The presence of carbon in the plasma is known to affect the ionization efficiency of As and it is also known to create a spectral overlap, $^{40}Ar^{12}C$, for ^{52}Cr. Our approach adds ethanol to all preparations (blanks, calibrators, QC and samples) to normalize the non-spectral interference of carbon on arsenic across urine samples which have varying levels of diluteness. However, the presence of ethanol in the reagents adds to the spectral interference of $^{40}Ar^{12}C$ on ^{52}Cr. Because chromium concentrations in urine for the normal population are expected to be low(Komaromy-Hiller et al., 2000; Roduchkin and Odman, 2001), we needed to change our approach for analysis of chromium from the use of helium gas in KED mode to the use of ammonia gas in DRC mode. Because the NexION 300 platform used here has two cell gas channels, we also needed to change our approach for analysis of arsenic from 10% hydrogen in argon in DRC mode, to helium gas in KED mode, along with nickel. Here we describe validation of these approaches within the BioUCell.

Reports of removing spectral interferences with ICP-MS collision/reaction cell instruments for measurement of As, Cr, and Ni in urine have been published, but none address our specific concerns for applying the method for biomonitoring measurements. NH_3 gas in an ICP-MS reaction cell was reported as effective at removing interferences on ^{52}Cr in a urine matrix(Chang and Jiang, 2001; Nixon et al., 2002). However, these reports do not discuss NH_3 effectiveness in an application where a carbon source, i.e. an interfering substance, is required for the benefit of another analyte. Choe(Choe and Gajek, 2016) describes a multi-element ICP-MS method for urine samples (including Cr and As, but not Ni) with the addition of an organic solvent to improve As ionization, however, the method uses He gas in a collision (octopole) cell for both As and Cr. Brodzka(Brodzka et al., 2013) published a method for measurement of As, Ni, Cr in urine by ICP-MS, but does not add a carbon source for As. Additionally the method measures Cr and Ni with methane gas, and uses oxygen gas for As. Our literature search resulted in evidence for analyzing As in a collision cell with only He or H_2/He mixtures on ICP-MS instruments equipped with octopole (Heitland et al., 2017; Leonhard et al., 2002; Nakazato et al., 2002) and hexapole (Dufailly et al., 2008; Li et

al., 2014; Niemelä et al., 2003) collision cells, but not with a quadrupole based collision cell as we have used.

Our laboratory has been measuring arsenic in urine by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) for 16 years as a part of the NHANES survey(Caldwell et al., 2009). In 2017, Ni and Cr were included in the NHANES survey for the first time, measured along with As per this method. Our method uses a NexION® 300D ICP-UCT-MS with a Bio-monitoring Universal Cell (BioUCell). The instrument has two reaction gas channels: channel A supplies anhydrous ammonia (NH_3) in DRC mode to remove primarily the $^{40}Ar^{12}C^+$ interference on $^{52}Cr^+$, and channel B supplies 100% helium (He) gas in Kinetic Energy Discrimination (KED) mode to remove $^{40}Ar^{35}Cl^+$ interference from $^{75}As^+$ and $^{44}Ca^{16}O^+$ interference from $^{60}Ni^+$. Urine samples are diluted 1:10 with 2% (v/v) HNO_3, 1.5% (v/v) ethanol, and 10 μg L^{-1} Rh (internal standard). Rinse solution is the same as the diluent with 0.002% Triton™ X-100 added and without internal standard. External, matrix-matched, weighted-linear ($1/x^2$) calibration is used to quantify total elemental concentration of As, Cr, and Ni in human urine samples.

2. Materials and methods

2.1. Instrumentation

A NexION® 300D ICP-MS with a BioUCell universal cell technology (UCT) upgrade (PerkinElmer Inc., Shelton, CT) was used and operated with the Syngistix™ computer software package v2.2 (PerkinElmer Inc., Shelton, CT). The ICP-MS was equipped with a Poly-Pro ST microflow concentric nebulizer with a 0.25 mm i.d. liquid capillary (Elemental Scientific Inc., Omaha, NE), concentric quartz spray chamber, demountable quartz torch, 2.0 mm i.d. quartz injector, platinum (or nickel) sampler and skimmer cones, and aluminum hyperskimmer cone. Instrumental parameters are listed in Table 1. We used an SC4-DX autosampler (Elemental Scientific Inc., Omaha, NE) connected to a FAST DXi-Integrated module (Elemental Scientific Inc., Omaha, NE) which replaced the standard peristaltic pump. These two components transferred prepared samples from the autosampler racks to the nebulizer. Details on the FAST valve components used, and our FAST program can be found in the supplemental section (Supplemental Tables 1 and 2). The autosampler was covered with a clear acrylic enclosure to prevent sample contamination with an ULPA filter to vent any caustic fumes from inside the enclosure. The UCT was supplied with >99.99% anhydrous ammonia (Matheson Tri-Gas, Montgomeryville, PA) and 99.9999% research grade helium (Airgas South, Atlanta, GA). The helium gas passed through an advanced filter system (PerkinElmer, Shelton, CT) to remove extraneous oxygen, water, and hydrocarbons prior to entering the UCT. UCT settings are listed in Table 2. The Cell Gas values listed in Table 2 are the numerical values entered in the software and while they are representative of the gas flow rate entering the UCT, they are not the true gas flow rate in mL min^{-1}. A Hamilton MicroLab® 600 dual-syringe diluter (Reno, NV), equipped with 10,000 μL diluent syringe and a 1000 μL sample syringe was used to prepare dilutions for analysis. The diluter was operated inside a Class II type A/B biological safety cabinet (BSC) (Nuaire, Plymouth, MN, USA).

2.2. Materials and reagents

We prepared all aqueous reagents with ≥18.2 MΩ cm deionized (DI) water from a Milli-Q® Advantage A10 water purification system (EMD Millipore, Billerica, MA). We used Veritas™ double distilled (DD) grade nitric acid (HNO_3), environmental grade HNO_3, and Veritas™ double distilled (DD) hydrochloric acid (HCl) from GFS Chemicals Inc. (Columbus, OH). USP dehydrated 200 proof ethanol was purchased from Pharmco Products Inc. (Brookfield, CT) and Triton X-100™ was purchased from J.T. Baker Chemical Co. (Center Valley, PA). Elemental stock solutions traceable to Standard Reference Materials (SRMs) made by the National Institute of Standards and Technology (NIST) were purchased: 1000 μg mL^{-1} As in 2% (v/v) HNO_3, and 100 μg mL^{-1} Cr and Ni in 2% (v/v) HNO_3 from High Purity Standards (Charleston, SC), 1000 μg mL^{-1} Rh from SPEX Industries, Inc. (Edison, NJ), 10,000 μg/mL Ca, and 1000 μg/mL Fe from Inorganic Ventures (Christiansburg, VA).

Reference materials used for method accuracy included NIST SRM 2668 (Gaithersburg, MD), and historical proficiency testing (PT) program samples from the Multielement External Quality Assessment Scheme (QMEQAS) of the Center de toxicologie du Québec (CTQ) (Quebec, Canada), and the urine trace elements (UTE) program of the Wadsworth Center (Albany, NY). Human urine was collected from anonymous donors under Institutional Review Board (IRB) approval to be used as the base matrix for calibration standards. Each urine donation was pre-screened and selected to be low in concentration of method analytes (see Table 3). Samples, calibration standards, and blanks were diluted into 15 mL polypropylene conical tubes (Greiner Bio-One North America Inc., Monroe, NC). All sample preparation and collection devices were pre-screened to be free of significant analyte contamination as described in previous publications(Jones et al., 2017; Ward et al., 2018).

2.3. Matrix-matched calibrator and urine sample preparation

Stock elemental standards were diluted with 2% (v/v) DD HNO_3 in acid-washed, Class B polypropylene volumetric flasks to prepare six intermediate working calibration standards (see Table 3). A portion of the 2% (v/v) DD HNO_3 was saved to prepare the matrix blank. Matrix-matched working calibrators (S1–S6) were prepared by mixing an aliquot of each intermediate working calibrator with base urine and diluent using the MicroLab® 600 (see Table 3). The matrix blank (S0) was prepared in the same manner using the 2% (v/v) DD HNO_3. Urine samples (including QC) were prepared at a 10× dilution by mixing an aliquot of each sample with diluent. The reagent blank (also called the aqueous blank) was prepared similarly, replacing the urine volume with ≥18.2 MΩ cm water, and was used as the blank for all patient samples, urine quality controls, and reference materials.

2.4. Sample preparation for ethanol optimization

Sample preparation of urine specimens was performed by a simple dilution of 0.5 mL urine with 4.5 mL of diluent (2% (v/v) HNO_3 and 10 μg L^{-1} Ga internal standard with 0–2.5% of ethanol). Matrix-matched calibrators were prepared by adding 0.1 mL of a calibration standard into 0.9 mL of pooled urine (normal base urine, tested to be < 5 μg L^{-1} As) and 9 mL of diluent. For the standard addition method to determine the “true” value of a sample, a

dilution of 0.25 mL urine, and 0.25 mL standard solution with 4.5 mL of diluent was used. Sample dilutions were accomplished using a Micromedic Digiflex™ automatic pipette (Titertek, Huntsville, AL).

3. Results and discussion

3.1. Ethanol addition to normalize ionization of As

After publication of Jarrett's method for measuring As (i.e. total arsenic concentrations) in human urine(Jarrett et al., 2007), we added ethanol to our sample diluent to improve method accuracy, and improve agreement of total arsenic results with the sum of species results reported from a CDC method determining seven arsenic species in urine (Verdon et al., 2009). Though the previous total arsenic method already included base urine in calibrators to matrix-match against urine QC and patient samples, ethanol addition into the diluent of all preparations normalizes the carbon enhanced ionization of As in the plasma(Larsen and Sturup, 1994) when a wide range of urine matrices exist across blanks, calibrators, QC, and patient samples. We tested 0.5% (v/v), 1.5% (v/v), and 2.5% (v/v) ethanol in the diluent, comparing total arsenic results for 32 urine samples from these external, matrix-matched calibrations to those results obtained by standard addition determinations. Standard addition determination for each sample included a spike at 0 μg L^{-1} As and two other standard levels (25 μg L^{-1}, 50 μg L^{-1}, 75 μg L^{-1}, 150 μg L^{-1}, 300 μg L^{-1}) based on the As concentration in the sample. Total arsenic results from 1.5% to 2.5% ethanol addition were in best agreement with the values from the standard addition experiments. We chose to use 1.5% ethanol since it was a lower cost option. Jarrett et al.[5] previously noted that addition of ethanol at 1–2% in the diluent increased observed measurement standard deviations by a factor of 3–4 times. However, in this method, measurement precision when using 1.5% ethanol in the diluent is ~1% RSD as observed in our characterized bench QC performance (Supplemental Table 3).

3.2. Elimination of cross-talk with the BioUCell

During method development we observed cross-talk in our internal standard, Rh, in DRC mode. Although the Rh concentration was constant in all samples, we measured a higher Rh signal in samples with a larger total ion concentration compared to signal measured in samples with a lower total ion concentration. Four urine samples we frequently measure during method development are, a low QC pool, a high QC pool, NIST SRM 2668 L1, and NIST SRM 2668 L2. We do not know the true total ion concentration of these samples, so we calculated an approximate, relative ion concentration for these samples by summing the concentrations of 19 elements listed on the NIST SRM certificate that we have also measured in our low and high QC pools (see Table 4). We analyzed each of these samples in DRC (for Cr with Rh as internal standard) and KED (for Ni and As with Rh as internal standard) modes with the AFV set to 325 V in the original NexION UCT cell. The Rh signals measured in the high QC pool and the NIST SRM L2 samples were consistently larger than the signals measured in the low QC pool and NIST SRM L1. In Table 4 we've presented this data as the average percent recovery of Rh signal relative to the low QC pool. The cross-talk appears as ≥ 110% recovery of the Rh signal. This behavior is not a matrix effect occurring in the plasma because we do not see the same behavior in KED mode. It is also not a hysteresis phenomenon(Bandura et al., 2002; Morris et al., 1994) where ion signal

is trapped from a previous mass scan, because the Rh signal measurements are very precise, average ≤ 1.4% RSD (see Table 5), even when the cross-talk is occurring. Increasing the AFV to 475V on the UCT did initially improve $^{103}Rh^{+}$ recovery to within 10% of the expected signal, but not permanently. We found that the phenomenon would return with instrument usage over subsequent weeks, and eventually we were not able to increase the AFV further (max value is 498 V). This phenomenon is not particular to Rh, or to internal standards. The Rh behavior was what brought this to our attention because we expect a to see a consistent signal for the internal standard during an analytical run (it is spiked into the diluent used to prepare all blanks, calibrators, QC, and samples). Over-recovery of Rh signal caused a negative bias in our measurements of $^{52}Cr^{+}$. In NIST SRM 2668 L2 the Cr target value is 27.7 μg L^{-1}, and at an AFV of 325 V our average measured Cr concentration in this sample was 15.8 μg L^{-1}.

Both Bandura(Bandura et al., 2000, 2002) and Tanner(Tanner et al., 2002) acknowledge that space charge effects will influence ion transmission and stated the Axial Field Voltage (AFV) is supposed to correct this behavior. This cross-talk behavior could not be replicated on an ELAN DRC II instrument, implying a difference between the cell design of these instruments. The BioUCell, has corrected crosstalk and has provided consistent performance in our lab. Table 4 lists the percent recoveries for Rh with the BioUCell just after installation with an AFV = 250 V. We now operate the instrument at AFV = 400 V but that value has been stable for more than two years. We have previously presented our observations of this phenomenon affecting another method in our lab running on a NexION ICP-UCT-MS(Jones et al., 2014), but the method procedure described here was the first in our lab to benefit from the BioUCell.

3.3. Internal standard selection for ^{75}As

We selected ^{103}Rh as the internal standard to correct for instrument drift and matrix-effects for all three analytes, ^{75}As, ^{60}Ni, and ^{52}Cr. Quarles et al. (2014) used ^{103}Rh as the internal standard for ^{52}Cr and ^{60}Ni, but Jarrett et al.[1] used gallium (^{71}Ga) as the internal standard for ^{75}As. Colon et al. (2011) demonstrated Ga, Ge, Y, and Rh are all acceptable internal standards for ^{75}As in the presence of a heavy matrix. We selected ^{103}Rh over ^{71}Ga as the internal standard for ^{75}As to simplify the reagent mixtures. This selection prevents the accidental usage of ^{69}Ga as the internal standard for As. ^{69}Ga has an interference from $^{138}Ba^{++}$, an element known to be in human urine samples(U.S. Department of Health and Human Services Centers for Disease Control and Prevention).

3.4. UCT conditions to remove polyatomic interferences

Table 6 contains a list of problematic spectral interferences for As, Cr, and Ni from elements we expect to find in high concentrations in urine: C, Cl, Ca, and Fe, combined with each other or with atmospheric or plasma gases. Table 6 also contains reference values for these elements in human urine. A full list of all possible spectral interferences can be found for ^{52}Cr and ^{60}Ni in Quarles et al. (2014) and in the supplemental section for ^{75}As (see Supplemental Table 4).

Selection of UCT gas to remove polyatomic interferences on $^{75}As^{+}$ and $^{52}Cr^{+}$ was limited because the NexION 300D instrument has two UCT gas channels, one of which must supply He gas for reduction of $^{44}Ca^{16}O^{+}$ on $^{60}Ni^{+}$ (Quarles et al., 2014). We investigated using He gas in KED mode, and 10% H_2/90% Ar, and O_2 gases in DRC mode for both $^{75}As^{+}$ and $^{52}Cr^{+}$. Additionally, we investigated NH_3 gas in DRC mode only for $^{52}Cr^{+}$ because it is known to react with $^{75}As^{+}$(Tanner et al., 2000). We measured $^{75}As^{+}$ and $^{52}Cr^{+}$ in NIST SRM 2668 Levels 1 and 2 after selecting UCT gas flow rates and RPq values through software optimizations. Table 7 lists the calculated percent bias from the certified target values for $^{75}As^{+}$ and $^{52}Cr^{+}$, for the conditions we tested. All UCT gases resulted in similarly accurate measurements for As with percent biases ≤ ±7% for both NIST SRM levels. NH_3 was the only gas that resulted in a percent bias of ≤20% for $^{52}Cr^{+}$ in the level 1 SRM, but at the higher concentration SRM level 2, any of the gases demonstrated acceptable performance. There is a notable difference in the biases measured for Cr in SRM level 1 using He, 10%H_2, or 100% O_2 gases compared to NH_3 gas. The measurements in KED mode and with 10% H_2 gas were made with 1.5% ethanol in the diluent, but we don't believe the high (≥50%) measurement bias is due to incomplete removal of $^{40}Ar^{12}C^{+}$ at m/z 52, but instead due to the very low sensitivity for 52Cr (<200 cps/μg L^{-1}) at the conditions required to remove the $^{40}Ar^{12}C$+ interference. The ^{52}Cr measurements with O_2 gas were made without ethanol in the diluent, and the bias in level 1 (92%) was surprising because there is evidence O_2 gas removes interferences on ^{52}Cr (Ambushe et al., 2009; Quarles et al., 2014). We observed a similar bias in measurements of ^{52}Cr in our low QC pool, which is also a multi-element urine matrix sample. We were not able to identify the source of the bias. NH_3 is the only acceptable UCT gas to further test for $^{52}Cr^{+}$, and because it is not a viable UCT gas for As determinations, we had to select KED mode with He gas for both Ni and As.

The NIST SRMs materials are a human urine matrix and presumably contain elements that could create spectral interferences for ^{75}As, ^{52}Cr, and ^{60}Ni, such as C, Cl, Ca, and/or Fe. In Table 6 we listed the polyatomic ions of concern for our analytes, which all stem from C, Cl, Ca, and/or Fe, and the concentrations of these elements in human urine we found in the literature. These elements are all essential for human health, which means they will be present in all urine samples, and in a wide range of possible concentrations.

We determined the ^{75}As and ^{52}Cr background equivalent concentration (BEC) in the vented and UCT modes from high concentration solutions of C, Cl, Ca, and/or Fe (see Table 8). The vented mode BEC proves the interference forms in the plasma and could interfere with analyte quantification, and the UCT mode BEC, reduced or removed relative the vented mode, is proof the UCT conditions are effective. The concentrations of C, Cl, Ca, and Fe we tested are listed in Table 8. We tested the C interferences at the concentration of ethanol in sample diluent, 1.5%. We selected a much higher Fe concentration than what is listed in the literature due to concern about Fe supplements and samples containing red blood cells. For this experiment individual As, Cr, and Ni calibration standards were prepared. As and Ni standards were diluted in 2% (v/v) DD HNO_3 and 1.5% ethanol, but no ethanol was present in the Cr calibration standards. Samples prepared at the concentrations in Table 8 were also in a 2% (v/v) DD HNO_3 matrix.

The vented mode BECs for ^{75}As ranged from 0.343 to 10.8 μg L^{-1}, all of which were reduced using the UCT in KED mode with He gas value set to 4.5. The solutions containing HCl and HCl + Ca created significantly larger As BECs in vented mode, 3.81 and 10.8 μg L^{-1}, respectively, but were reduced to 0.16 and 0.18 μg L^{-1}, respectively in KED mode. There was only a small reduction in the BEC from the Fe solution from 0.343 to 0.229 μg L^{-1}. KED mode is an effective interference removal technique because polyatomic ions undergo a higher number of collisions with the He gas than the analyte ion. If KED mode can reduce the larger ion signals from $^{40}Ar^{35}Cl^{+}$ and $^{40}Ca^{35}Cl^{+}$, as evidenced by the data in Table 8, it should be effective in removing ion signal due to $^{58}Fe^{16}O^{1}H^{+}$and/or $^{56}Fe^{18}O^{1}H^{+}$. This could indicate that most of the elevated BEC from Fe in vented mode and all the residual As BECs in KED mode is actually due to As contamination in the solutions. The vented mode BECs for $^{52}Cr^{+}$ ranged from 2.85 to 789 μg L^{-1}, all of which were reduced to background levels with NH_3 as the UCT gas. During this experiment we only measured $^{60}Ni^{+}$ in the presence of Ca in the pressurized UCT (KED mode). We measured the $^{60}Ni+$ BEC at background levels, indicating the $^{44}Ca^{16}O^{+}$ interference was reduced. We've listed the vented mode BEC from our previous work on the same instrument type for comparison.

The pressurized UCT mode BECs in Table 8 were measured after the BioUCell was installed on our instrument. An unexpected advantage of the BioUCell is a reduction in the cell gas flow rates required to reduce interference signals, in both DRC and KED modes, compared to the flow rates determined for the original UCT. The NH_3 gas value for Cr analysis was reduced from 1.0 to 0.7, and the He gas value for Ni analysis was reduced from 6 to 4.5. We had been measuring As at a He gas value of 4.5 in the original UCT cell and after the BioUCell was installed we did not reduce this value, and opted to use the same gas flow setting for As and Ni in KED mode for faster sample throughput. However, as we evaluated As data for the LOD calculation at a He value of 4.5, we realized the low sensitivity for As was going to result in a higher LOD than our previous method. We opted to reduce the He setting to 2.5 for As which resulted in a factor of 16 increase in the As signal. This decision added time to the method (~30 s) but allowed the precision necessary to avoid a higher LOD. Final UCT conditions were listed in Table 2.

3.5. Accuracy

We verified method accuracy by analysis of NIST Standard Reference Materials (SRM). NIST SRM 2668 "Toxic Elements in Frozen Human Urine" has certified concentrations for As in Levels 1 and 2, for Cr and Ni in Level 2, and reference concentrations of Cr and Ni in Level 1. This SRM was measured more than 20 times during method validation, on two instruments, and by three analysts. Table 9 lists our measured mean concentration, 95% confidence interval, and percent bias relative to the target value. Only Cr in 2668L1 sample is not repeatedly measured within 4% of the target value; the average bias is +13%, however our 95% CI overlaps the range of the target value + *k*, 0.77 μg L^{-1} –1.39 μg L^{-1}. The average biases for As, Cr and Ni across both NIST SRM levels are −1.5%, 8.3%, and 1.1%, respectively. Also listed in Table 9 is the percent bias for As, Cr, and Ni in the NIST SRMs from each of the previous methods. Comparing the accuracy of the previous methods to the method described here demonstrates we did not significantly sacrifice accuracy for lab efficiency.

3.6. Precision

Method reproducibility is reflected in the statistical characterization of bench QC pools from more than 50 analytical runs. As, Cr, and Ni concentrations were measured in three QC pools (low, high, and elevated) and the means, standard deviations, and %CVs are listed in Table 10. All pools were measured with percent CVs between 3% and 6% except for the low pools for Ni and Cr which both have percent CVs of 12%. Fig. 1 shows the characterized bench QC limits of the new, combined method and the old methods as a direct comparison of the method precisions. The new method has better, or comparable, precision for Cr and Ni. The new method precision for As is approximately twice as large, but still represents less than 5% CV. During the characterization process, we rotate calibration standard manufacturing lots (N = 3), include work by multiple laboratory analysts, perform routine maintenance on the instruments (e.g. sample introduction parts and cones are either replaced, or cleaned and reinstalled), and use multiple instruments to capture method variation anticipated to be observed during normal method implementation in our laboratory. This amount of variation during the QC characterization period adds ruggedness to our QC limits. The full QC characterization statistics (mean, STDc, STDi, and STDw) are listed in Supplemental Table 5. The multi-rule quality control system (MRQCS) developed by Caudill et al. (2008) was used to establish statistical characteristics for each bench QC pool and used to determine if each analytical run was in control.

Our laboratory information system (LIMS) is programed to flag results whose intra-measurement concentration range exceeds a maximum permitted concentration range (max-min) of the three replicate readings for a single sample measurement. The maximum permitted range is a two-phase intra-measurement precision limit imposing a fixed limit at lower concentrations and a percentage-based limit at higher concentrations: for As 2 μg L^{-1} for values < 20 μg L^{-1} and 10% of the value at ≥20 μg L^{-1}, for Cr 0.4 μg L^{-1} for values < 4 μg L^{-1} and 10% of the concentration ≥ 4 μg L^{-1}, and for Ni 0.5 μg L^{-1} for values < 5 μg L^{-1} and 10% of the value at ≥ 5 μg L^{-1}. We have observed range maximum failures for all three elements, but the failure rate is quite low. Out of ~6500 measurements for As, and ~5500 measurements for Cr and Ni (each), the percentage of measurements that failed this measure are 0.03%, 1.58%, and 0.85% for As, Cr, and Ni, respectively. When a failure occurs, the results from the measurement are not reported; the sample is re-prepared and remeasured.

We have observed intra-measurement precision failures for ^{52}Cr where the signal from one replicate measurement is significantly larger (up to 130,000 cps) than the remaining two. We refer to these occurrences as Cr spikes. The magnitude of the spikes observed for Cr are larger than any observed for As or Ni. This issue is not related to sample inhomogeneity (i.e. an undissolved particle reaching the plasma) because spikes are observed without the urine matrix present. The source of the signal is still undetermined, but the signal is thought to be actual chromium ions since it was observed at both ^{52}Cr and ^{53}Cr during troubleshooting tests. The spikes were not eliminated by replacing hardware including the ammonia cylinder, the ammonia regulator, the stainless-steel tubing connecting the ammonia cylinder to the ICP-MS, the mass flow controllers, or the BioUCell, and spikes are observed on two separate NexION systems each connected to individual cylinders of NH_3. The spikes were not eliminated by including ^{50}Cr into the method file which would be analyzed immediately

prior to the ^{52}Cr ion, ruling out a potential artifact from long-distance peak hopping prior to analysis. We have noticed a reduction in the frequency and magnitude of the spikes after installation of Syngistix™ 2.2, which allows us to fix the sequence of DRC and KED modes by running different RF power settings for DRC (1599W) and KED (1600W) modes. This forces the software to execute the lower RF power mode first (DRC mode) and allows a longer amount of time for the ammonia flow rate to stabilize in the cell. The instrument switches to DRC mode during the sample flush and read delay which allows NH_3 to begin flowing to the reaction cell. This equals a longer length of time than the typical 30 s delay to pressurize the reaction cell. Previous versions of the NexION or Syngistix™ software only use a single setting of RF power for every mode and will only execute analysis in the following order 1) KED mode 2) DRC mode 3) standard mode.

3.7. Extra sample dilution with water

We performed experiments that tested extra dilutions of urine samples up to 20× with ≥18.2 MΩ cm^{-1} water. We selected six samples (NIST SRM 2668 L2, elevated bench QC, two PT samples from each CTQ and the Wadsworth Center programs) to use in this experiment. Each of the six samples were prepared for analysis with the following extra dilution factors, 1× (i.e. no extra dilution), 2×, 5×, 10×, and 20×, for a total of 30 results (six samples, five dilutions). The measured concentrations of samples with extra dilutions were multiplied by the dilution factor to determine the final result and then normalized to the measured concentration with no extra dilution (see Supplemental Table 6). The average normalized concentration and standard deviation (per extra dilution factor) were calculated and are summarized in Table 11.

Concentrations of As and Cr measured in samples with extra dilutions results were under recovered with average normalized values <1.00. In addition As and Cr results exhibited a decreasing recovery trend with increasing extra dilution factor (from 2× to 20×), as 0.97 to 0.91 for As, and 0.98 to 0.91 for Cr. Ni final results at 2×, 5×, and 10× were similar to the observed result with no extra dilution (±0.03 of the 1× results), but at a 20× dilution factor the recovery dropped to 0.90 of the no dilution result. The standard deviations of the As averaged normalized final results are very small, 0.01 or 0.02, indicating the low recovery is independent of sample matrix. Ni and Cr results were much more varied at each dilution level (i.e. larger SDs).

Extra dilutions of biological sample matrices in ICP-MS can result in a bias in the final sample concentration because modifying the matrix may interfere with matrix-matched calibration. The data in Table 11 demonstrate that, on average, normalized final results with extra dilutions up to 20× are within ±10% of the no extra dilution observed results. However, the calculated standard deviations indicate there is a significant amount of variability in the measurements, primarily for Cr and Ni, and statistically, a 20× extra dilution factor would occasionally cause a final result to be more than −10% of the concentration measured without extra dilution. The negative bias gives rise to concerns about reporting a ‘false negative’ result back to a patient (i.e. a final result that is lower than the true value and may appear to be in the normal human population range). Extra dilutions are only performed when an initial observed result is above the highest calibration standard, 1500 μg L^{-1} for As,

and 125 μg L^{-1} for Cr and Ni (see Table 3). At 20× these concentration levels a −10% bias would likely not result in incorrect clinical treatment. However, data from our laboratory has other implications beyond patient care. In biomonitoring studies such as NHANES, the data is used to set population reference ranges and we regularly participate in reporting several proficiency testing samples each year. We concluded this experiment by adopting a conservative practice that when necessary, urine samples can be diluted up to and including an extra 5× dilution, but the lowest dilution factor possible should always be used.

3.8. Calibration range, limit of detection and sample washout

The concentrations of calibration standards (S1–S6) for As, Cr, and Ni can be found in Table 3. We selected the weighted linear regression ($1/x^2$) option in the Syngistix™ software to maintain accuracy at the low end of the calibration curve where we typically measure biomonitoring samples. As part of method validation, we scrutinize our calibration curve data for non-linearity through examination of residual plots, calculation of the residual sum of squares, and quadratic regressions; no curvilinear behavior was found. The concentration of the first calibration standard for As is an order of magnitude lower in this method compared to Jarrett et al. (2007), 0.2 μg L^{-1} vs 2.0 μg L^{-1}, respectively. We also increased the highest calibrator for all elements, from 10 μg L^{-1} to 125 μg L^{-1} for Ni and Cr, and from 200 μg L^{-1} to 1500 μg L^{-1} for As. The extended calibration range and the validated 5× extra dilution factor increases the maximum reportable range to 7500, 625, and 625 μg L^{-1} for As, Cr, and Ni, respectively (see Table 12).

Method limits of detection (LOD) were calculated in a manner equivalent to the recommendations by the Clinical Laboratory Standards Institute (CLSI) which includes both Type I and Type II error estimates (CLSI, 2012). We measured a matrix blank and three low-concentration spiked urine pools in at least 60 runs over a two-month timeframe. Pool concentrations were 0.2, 0.6, and 4 μg L^{-1} for As, and 0.2, 0.4, and 2 μg L^{-1} for Cr and Ni. The mean and standard deviation for each blank and spike pool was calculated and plotted against each other for each analyte. The calculations used to determine the LOD concentration were described by Georgi(Georgi et al., 2017). Our calculated method LODs for As, Cr, Ni are listed in Table 11 along with the upper reportable range of our method, the highest calibrator concentration multiplied by the allowed extra dilution factor. We included in this table population reference ranges from NHANES(U.S. Department of Health and Human Services Centers for Disease Control and Prevention), Health Canada biomonitoring study, and recent ranges used in proficiency testing challenge samples for clinical laboratories to demonstrate that our method is appropriate for both biomonitoring applications and to assess acute exposures.

We validated a 30 s rinse time was sufficient to prevent signal carryover. We analyzed the highest concentration standard followed by preparations of our low QC pool. The measured concentrations of the low QC pool sample was within the characterized limits even after 5 sequential measurements of the high concentration standard indicating no signal carryover occurred.

4. Conclusions

We developed an ICP-UCT-MS method to quantify As, Cr, and Ni in human urine samples, replacing two existing methods with one to improve laboratory efficiency. The analyte combination presented challenges to method accuracy and precision but use of the BioUCell and appropriate selection of reaction/collision gas resulted in a method for use in biomonitoring applications, such as the NHANES survey. Starting with the 2017–2018 cycle, this will produce all NHANES results for As, Cr, and Ni in human urine. In addition, we increased the highest calibrator of all elements to include concentrations which might be observed as a result of acute exposures, making it a method also designed for emergency response situations. Our rigorously calculated limits of detection are similar to our previously published methods (Jarrett et al., 2007; Quarles et al., 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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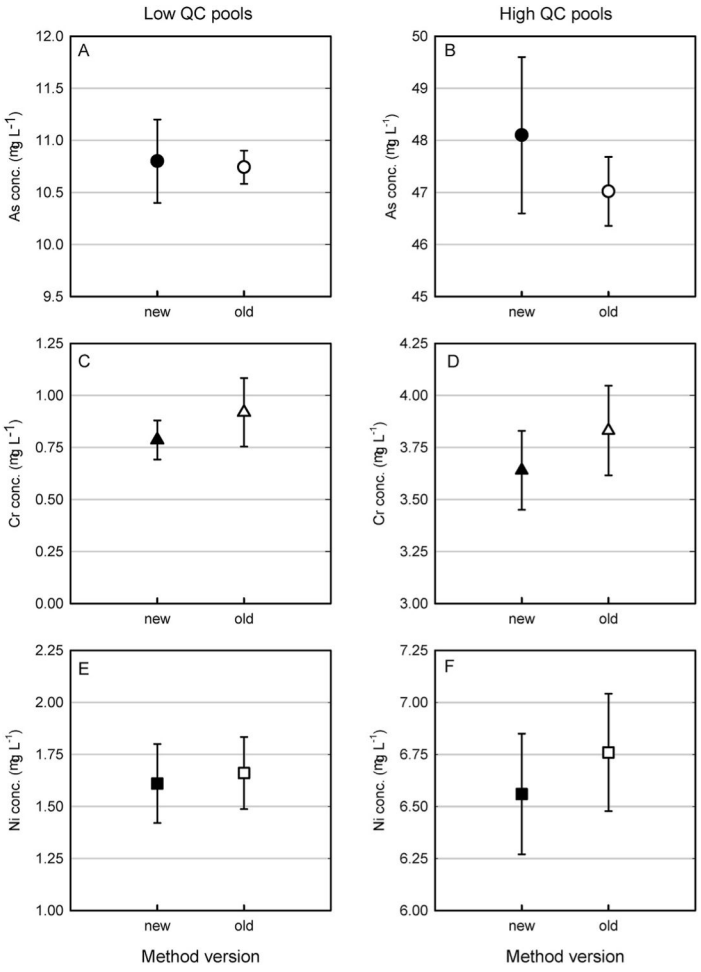

Fig. 1.

Comparison of characterized QC limits for new method (closed symbols) and old method (open symbols). Error bars represent 1SD of the characterized mean.

Table 1

Instrument parameters for the PerkinElmer NexION™ 300D ICP-MS.

Instrument parameter	Value/setting
RF power	DRC: 1.599 kW KED: 1.600 kW
Plasma gas flow (Ar)	18 L min^{-1}
Auxiliary gas flow (Ar)	1.2 L min^{-1}
Nebulizer gas flow (Ar)	~0.8–1.1 L min^{-1}
Scan mode	Peak hopping
Sweeps/reading	70
Readings/replicate	1
Replicates	3
Dwell times	150 ms for ^{60}Ni, 100 ms for ^{75}As 70 ms for ^{52}Cr 30 ms for internal standard ^{103}Rh
Quadrupole Ion Deflector	On; [STD/DRC] QID and [KED] QID
Detector mode	Dual
Calibration Regression Type	External, matrix matched, weighted linear[a]
Sample Flush	9 s
Read Delay	45 s
Rinse time	30 s
Enable QC Check	On
Sample QC	Upper concentration limits: Ni/Cr 125 μg L^{-1} As 1500 μg L^{-1} Action 1: Wash for X and Continue Action 1 Data: 200 s
Pressurize delay	40 s
Exhaust delay	40 s
Channel delay	40 s
DXi-FAST pump speed (liquid flow rate)	3 rpm (310 μL min −1 with 0.76 mm i.d. tubing)

[a] The Syngistix™ software uses a $(1/x^2)$ weighting.

Table 2

Analyte, internal standards, equations, and DRC/KED parameters. Syngistix™ software version 2.2

Isotope	Internal Standard	Equation	UCT Mode, Gas channel	Gas	Cell Gas (A or B) value[a]	Rpq	Rpa	KED[b] (V)	AFV[c]
^{52}Cr	^{103}Rh	None	DRC, Channel A	NH_3	0.7	0.6	0	n/a	400
^{75}As	^{103}Rh	None	KED, Channel B	He	2.5	0.25	0	3	475
^{60}Ni	^{103}Rh	None	KED, Channel B	He	4.5	0.25	0	3	475

[a]Numerical value used in the software.

[b]KED = QRO – CRO.

[c]Typical value, optimize per instrument.

Table 3

Reagents, sample composition, and calibrator concentrations.

Reagent name	Composition
Diluent and FAST carrier solution[a]	2% (v/v) HNO_3, 1.5% (v/v) ethanol, 10 μg L^{-1} $_{Rh}$
Rinse solution[a]	2% (v/v) HNO_3, 1.5% (v/v) ethanol, 0.002% Triton X-100™
Intermediate working calibrators (μg L^{-1})[b]	As: 0.2, 1, 6, 30, 250, 1500 Cr and Ni: 0.1, 0.4, 2, 8, 30, 125
Matrix-matched calibrators (S1–S6)	60 μL intermediate working calibrator + 540 μL base urine + 5400 μL diluent
Matrix blank (S0)	60 μL 2% (v/v) HNO_3 + 540 μL base urine + 5400 μL diluent
Sample preparation	300 μL urine sample + 2700 μL diluent
Reagent blank	300 μL DI water + 2700 μL diluent
Maximum suggested base urine concentrations (μg L^{-1})	As: 5; Cr: 0.9; Ni: 1.5

[a] Double distilled grade HNO_3 is recommended for the diluent and carrier, but environmental grade can be used in the rinse preparation.

[b] A further 1:10 dilution occurs when added to base urine.

Table 4

Average recovery of Rh ion signal (relative to Low QC).

Sample ID:			**Low QC**[a]	**High QC**[a]	**NIST SRM 2668 L1**	**NIST SRM 2668 L2**	
Approx. ion concentration (μg L^{-1})[b]**:**			**87**	**463**	**112**	**3511**	
Cell type	UCT mode	AFV (V)	Rh signal recovery				N
Original UCT	DRC	325	100%	110%	101%	167%	3
Original UCT	KED	325	100%	101%	103%	102%	7
Original UCT	DRC	475	100%	100%	103%	101%	5
BioUCell	DRC	250	100%	99%	104%	103%	5
BioUCell	KED	475	100%	101%	104%	105%	5

[a]Pooled human urine spiked with elements of interest.

[b]Summation of concentrations of 19 elements (Sb, As, Ba, Be, Cd, Cs, Cr, Co, Cu, Pb, Mn, Mo, Ni, Pt, Tl, Sn, W, U, V); provided only as a relative comparison.

Table 5

Average intra-measurement precision of Rh signal (as %RSD).

Sample ID			**Low QC***	**High QC***	**NIST SRM 2668 L1**	**NIST SRM 2668 L2**	
Cell type	UCT mode	AFV (V)	Average Rh	%RSD			N
Original UCT	DRC	325	0.9%	1.4%	0.8%	1.0%	3

Table 6

Spectral overlaps for ^{75}As, ^{52}Cr, and ^{60}Ni from the urine matrix containing C, Cl, Ca, and Fe.

Analyte isotope	C	Cl	Ca	Fe
^{75}As	NA	$^{40}Ar^{35}Cl^{+}$ $^{40}Ca^{35}Cl^{+}$	$^{40}Ca^{35}Cl^{+}$	$^{58}Fe^{16}O^{1}H+$,$^{56}Fe^{18}O^{1}H^{+}$
^{52}Cr	$^{40}Ar^{12}C^{+}$, $^{40}Ca^{12}C^{+}$,	$^{35}Cl^{17}O^{+}$,$^{37}Cl^{15}N^{+}$,$^{37}Cl^{14}N^{1}H^{+}$,$^{35}Cl^{16}O^{1}H^{+}$	$^{40}Ca^{12}C^{+}$, $^{44}Ca^{16}O^{+}$	NA
^{60}Ni	NA	NA	30,000–600,000[b]	NA
Expected concentration in human urine (μg L^{-1})	~5,000,000[a]	200,000–18,000,000[b]	30–600[b]	25–101[c]; 0.67–331[d]
mg L^{-1}	5000	200–18,000[b]		0.025–0.101[c] 6.7×10^{-4} – 0.331[d]

[a] From Quarles et al.

[b] Ref. Burtis and Ashwood (1999).

[c] Ref. Moreno et al. (2010).

[d] Ref. Ivanenko et al. (2013).

Table 7

Accuracy of Cr and As measurements, as a percent bias from target value, with several UCT gases in DRC and KED modes.

Analyte	Sample ID	Certificate value (μg L^{-1})	KED: 100% He[a]	DRC: 10% H_2[b]	DRC: O_2[c]	DRC: NH_3[d]
As	NIST SRM 2668 L1	10.81 ± 0.54	−3.9%	−4.5%	−1%	N/A
	NIST SRM 2668 L2	213 ± 4.4	1.3%	−1.9%	7%	N/A
Cr	NIST SRM 2668 L1	1.08 ± 0.31	99%	53%	92%	18%
	NIST SRM 2668 L2	27.7 ± 2.1	4%	−3%	−6%	4%

[a] KED mode with 100% He gas: Cr at 6.0 and As at 4.5, RPq = 0.25.

[b] DRC mode with 10% H_2 in Ar gas: 1.8 for Cr and 0.7 for As, RPq = 0.6.

[c] DRC mode with 100% O_2 gas at 1.0, RPq = 0.7.

[d] DRC mode with 100% NH_3 gas at 1.0, RPq = 0.6.

Table 8

Background equivalent concentrations for ^{75}As, ^{52}Cr, and ^{60}Ni.

Analyte isotope	Concentration tested (mg L^{-1})	Vented UCT BEC (μg L^{-1})	Pressurized UCT[a] BEC (μg L^{-1})
^{75}As	HCl: 10,000	3.81	0.16
	Ca: 100	10.8	0.18
	Fe: 300	0.34	0.23
^{52}Cr	Ethanol: 15,000	609	0.00 (−0.007)
	Ethanol: 15,000 Ca: 100	789	0.00 (0.002)
	HCl: 10,000	2.85	0.00 (−0.014)
^{60}Ni	Ca: 100	3.64[b]	−0.02

[a] As and Ni in KED mode with He gas set to 4.5; Cr in DRC mode with NH_3 set to 0.7 and RPq = 0.6.

[b] From Quarles et al.

Table 9

NIST SRM 2668 Toxic elements in frozen human urine results from final method conditions.

SRM	Analyte	Average (μg L^{-1})	N	95% CI (μg L^{-1})	Certificate value ± U[a] (μg L^{-1})	% bias	% bias previous methods[b]
2668L1	As	10.5	34	10.4–10.6	10.81 ± 0.54	−3.3%	−4.3%
	Cr	1.22	29	1.15–1.29	1.08 ± 0.31	13%	16%
	Ni	2.33	34	2.29–2.37	2.31 ± 0.32	0.9%	−5.1%
2668L2	As	214	33	212–216	213.1 ± 44	0.3%	2.0%
	Cr	28.7	31	28.4–28.9	27.7 ± 2.1	3.6%	1.3%
	Ni	117	30	115–118	115.3 ± 5.2	1.3%	−0.3%

[a] U is the expanded uncertainty corresponding to approximately 95% confidence for each analyte.

[b] N = 12.

Table 10

Bench QC mean, standard deviation, and percent CV; data collected in >50 analytical runs; mean and SDs are in μg L^{-1}.

Analyte	Low Urine QC			High Urine QC			Elevated Urine QC		
	Mean	SD[a]	%CV[b]	Mean	SD[a]	%CV[b]	Mean	SD[a]	%CV[b]
^{75}As	10.8	0.4	3.97%	48.1	1.5	3.16%	1240	46	3.69%
^{52}Cr	0.786	0.094	12.0%	3.64	0.19	5.24%	59.6	2.6	4.39%
^{60}Ni	1.61	0.19	12.0%	6.56	0.29	4.45%	59.8	2.6	4.34%

[a] SD of run means (2 QC measurements per run).

[b] %CV calculated as = 100*(SD)/Mean.

Table 11

Average and standard deviation of the normalized extra dilution results.

	^{75}As	^{52}Cr	^{60}Ni
1× (no extra dilution)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
2×	0.97 ± 0.01	0.98 ± 0.02	0.99 ± 0.02
5×	0.95 ± 0.01	0.97 ± 0.04	1.02 ± 0.03
10×	0.92 ± 0.01	0.93 ± 0.02	1.03 ± 0.06
20×	0.91 ± 0.02	0.91 ± 0.07	0.90 ± 0.06

Table 12

Reportable range, biomonitoring statistics, and challenge concentrations from proficiency testing programs.

Source	As (μg L^{-1})	Cr (μg L^{-1})	Ni (μg L^{-1})
Method reportable range (LOD to cal 6 × 5)	0.23–7500	0.19–625	0.31–625
NHANES 2015–2016 (50th-95th) biomonitoring survey[d]	5.41–44.6	no data	no data
Health Canada 2009–2011 (50th-95th) biomonitoring survey[e]	7.8–76	no data	1.4–4.8
2017 Wadsworth Center proficiency testing (PT) program concentration ranges	5.6–560[a] 15.0–780[b]	0.85–29.6[a]	1.2–29.0[a]
2017 CTQ QMEQAS program[c] concentration ranges	27.8–192	1.62–57.2	2.07–47.5

[a]Urine trace elements program of the Wadsworth Center at the New York State Department of Health Trace Elements Laboratory.

[b]Laboratory response network toxic elements in urine program of the Wadsworth Center at the New York State Department of Health Trace Elements Laboratory.

[c]Quebec Multielement External Quality Assessment Scheme (QMEQAS) of the Center de toxicologie du Québec (CTQ) at the Quebec's National Institute of Public Health.

[d]Ref. U.S. Department of Health (2019).

[e]Ref. Health Canada (2019).