Analytical Methods

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Journal:	*Analytical Methods*
Manuscript ID	AY-ART-07-2016-001877.R1
Article Type:	Paper
Date Submitted by the Author:	16-Aug-2016
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Determination of trace elements in human urine by ICP-MS using sodium chloride as a matrix-matching component in calibration

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Abstract

An inductively coupled plasma mass spectrometry (ICP-MS) method was developed for the simultaneous determination of the following thirteen trace elements in human urine: Cr, Mn, Co, As, Se, Sr, Mo, Cd, Sb, Hg, Tl, Pb, and U. An Agilent 7700x ICP-MS fitted with a helium mode collision cell was used, along with an integrated sample introduction system for flow injection analysis. An optimal helium flow rate of 5.5 mL min^{-1} through the collision cell was determined based on background levels, analyte-to-background signal ratios, and analytical precisions. The sample diluent was added with n-butanol as a carbon source to enhance analytical sensitivity, which yielded higher signal intensities than methanol or ethanol. The basic diluent prepared with 4% (v/v) *n*-butanol, 1% (w/v) ammonium hydroxide, 0.1% (w/v) ethylenediaminetetraacetic acid (H_4EDTA), and 0.05% Triton X-100, also demonstrated excellent washout efficiency, requiring only < 60 sec of rinse time to minimize the Hg memory effect. Sodium chloride (0.95%, w/v) was added to intermediate calibration standards as a matrix-matching component in order to equalize analyte signal suppression caused by inorganic urine components. When both calibration standards and urine specimens were diluted 1:10 with the diluent, no considerable variability was observed in internal standard signals throughout the analyses, in spite of a wide range of Na content in urine specimens. Potential Mo oxide interferences on Cd signals were taken into account and minimized with empirically estimated correction factors. Analysis of standard reference materials as well as proficiency testing specimens yielded accuracies of 100 ± 10% for most certified/reference values. Long-term precision of the method was also routinely monitored with internal quality control urine specimens, and after a period of 60 days, the relative standard deviations were < 8% for all the analytes.

Introduction

Various biomonitoring programs evaluate endemic, occupational, and catastrophic exposures to toxic elements from various sources as well as the nutritional status of essential elements on an individual, population, or area basis.[1-4] Urine is the most commonly used specimen for biomonitoring studies as the method of sample collection is relatively simple and a variety of elements are excreted in urine as their original form or metabolites. Inductively coupled plasma mass spectrometry (ICP-MS) is a very sensitive and robust technique with a high sample throughput, and is widely employed for simultaneous determinations of multiple elements in urine specimens.[3-8] Accurate quantification of trace elements in urine by ICP-MS is, however, often challenging due to analyte concentrations at sub-μg L^{-1} levels and elevated levels of background constituents that may add up to ~37 g L^{-1} in typical human urine.[9] In addition, matrix elements including Cl (1.9 – 8.4 g L^{-1}), Na (1.2 – 4.4 g L^{-1}), and K (0.8 – 2.6 g L^{-1}) are closely associated with spectral and/or non-spectral interferences in ICP-MS analysis.

Spectral interferences are caused by single or polyatomic ions that have the same mass-to-charge ratio as the analytes of interest. These spectral interferences can relatively easily be minimized by various approaches: correction equations, collision/reaction cell technology, or high-resolution mass spectrometry. Non-spectral interferences, also known as matrix effects, involve the suppression or enhancement of analyte signal intensities in a sample solution, compared to those expected in a matrix-free solution. In contrast to spectral interferences, matrix effects are explained by numerous effects including solid deposition on nebulizer and cones, ICP cooling, changes in droplet size distribution, charge transfer from carbon ions to analytes, shifts in ionization equilibrium in the ICP, collisional scattering of ions, and space charge effects in the ion optics, etc.[10] Although isotope dilution mass spectrometry may be an ideal method to compensate for matrix effects as it can provide great precision and accuracy for high-matrix specimens such as urine[11-13], it cannot be applied to monoisotopic elements such as Mn, Co, and As. Standard addition methods can be an alternative, but such methods require a full calibration for a single sample and can be tedious. Pre-concentration of analytes by chemical separation of the matrix can be another great option to minimize matrix effects and to increase sensitivity. However, analyte-matrix separation techniques are generally time-consuming, require large

sample/reagent consumption, and are not appropriate for simultaneous measurement of a large list of analytes.

The most common approach to reducing matrix effects is the dilution of urine specimens with an acidic[3, 14-18] or basic[15, 19, 20] solution prior to analysis along with the use of internal standardization. Digestion procedures were occasionally employed[21] with oxidizing reagents such as concentrated nitric acid[5, 6] or a mixture of nitric acid and hydrogen peroxide.[22, 23] These "dilute and shoot" methods still result in significant discrepancies between instrumental responses for specimens and calibration standards due to considerable amounts of inorganic components. Furthermore, selection of a "good" internal standard (ISTD) for a given analyte based on first ionization potential and atomic mass in different matrices is sometimes difficult.[24, 25] Many studies added "base" urine to calibration standards to matrix-match urine specimens in order to resolve this discrepancy.[1, 5, 6, 14, 16, 19, 26] Applying urine as a matrix-matching component, however, can still cause potential biases that can be substantial if analytes of interest are present in the added urine.

Our recent studies suggested that "dilute and shoot" approaches could still be a reliable choice for simultaneous determination of Mn, As, Cd, W, Hg, Pb, and U in whole blood[27] and serum/plasma[28] specimens. In those studies, external calibration standard solutions were prepared with a mixture of NaCl and $CaCl_2$, or with NaCl only to match the signal suppression of analytes and ISTDs caused by matrix constituents in those biological fluids. Findings also demonstrated that carbon in the diluent maximized the signal enhancement effect as a result of the charge transfer from carbon ions (C^+) to analytes and ISTDs. In the present study, a similar ICP-MS method was developed based on using NaCl as a matrix-matching component in external calibrations for quantification of the following trace elements in human urine: Cr, Mn, Co, As, Se, Sr, Mo, Cd, Sb, Hg, Tl, Pb, and U. In contrast to the homeostatic nature of blood and plasma, levels of analytes and matrix components in spot urine can substantially vary depending on factors including a donor's eating habits and sample collection time, which may pose additional difficulties in accurate determination of these elements. Various operational and analytical conditions were evaluated to improve the sensitivity, accuracy, and precision. The washout efficiency of the basic diluent was also tested for Hg. The resulting method was applied

to determine all thirteen analytes in a minimal urine volume of 200 µL under identical analytical conditions. Because of analytical difficulties and difference in chemical stability of analytes in diluent, very few studies have tested this approach for a large list of trace elements including Hg.[26]

Experimental

Instrumentation - An Agilent 7700x quadrupole ICP-MS with a helium-mode octopole collision cell was used for this study. The ICP-MS was fitted with platinum sampler and skimmer cones, a MicroMist concentric nebulizer, and a chilled (2 °C) cyclonic quartz spray chamber (Glass Expansion KT1116Q). The ICP-MS was operated with integrated sample introduction system (ISIS) for flow injection analysis and interfaced with a CETAC ASX 500 series autosampler with a Teflon-lined sample probe that was placed inside an enclosure (CETAC ENC 500). Typical instrumental settings for the ICP-MS and ISIS are presented in Table 1.

The instrument was checked daily for sensitivity and interference levels. Instrumental sensitivity was determined using an instrument check solution containing 1 µg L^{-1} of ^{7}Li, ^{89}Y, and ^{205}Tl, which was typically greater than 80000, 200000, and 170000 counts per second (cps), respectively , in the absence of the collision gas. Formation of oxides and double-charge ions during analytical runs were estimated by monitoring $^{140}Ce^{16}O^{+}/^{140}Ce^{+}$ and $^{140}Ce^{2+}/^{140}Ce^{+}$, respectively. Both ratios were generally < 1.5 % in the absence of the collision cell gas. Isotopes and integration times selected for this study are listed in Table 1. For Pb, the sum of signal counts from three abundant isotopes, ^{206}Pb, ^{207}Pb, and ^{208}Pb, were used for quantification to avoid any potential bias caused by variability in isotopic ratios in urine. Average analytical results were calculated from triplicate measurements for each isotope using MassHunter version B.01.01 software.

Labware cleaning protocol – All brand-new polyethylene and Teflon containers used for preparation of reagents and standard solutions were cleaned with 10% HNO_3 at ~ 60 °C for > 8 hours, 2% (v/v) HCl at ambient temperature for > 48 hours, and then finally with 2% (w/v) NH_4OH with 0.1% (w/v) EDTA at ambient temperature for > 24 hours. All bottles were checked for contamination prior to use. Polypropylene centrifuge tubes with an internal volume of 10 mL

used for sample preparation were cleaned prior to use with a mixture of 2% (v/v) HCl and 1% (v/v) HNO_3 at ambient temperature for > 24 hours. Method blanks were checked daily and did not contain any detectable levels of the analytes of interest.

Calibration standards and reagents – All solutions needed for this work were prepared using deionized (DI) water (≥18.2 MΩ·cm resistivity) purified from a Millipore Milli-Q water system. Individual (Cr, Hg, and U) and mixed-element (Mn, Co, As, Se, Sr, Mo, Cd, Sb, Tl, and Pb) stock standards were purchased from SPEX CertiPrep (Metuchen, NJ) and Inorganic Ventures (Christiansburg, VA), respectively. Standard solutions were also purchased from a second source for calibration verification and were obtained from High-Purity Standards (Charleston, SC). A "synthetic-matrix" solution prepared for diluting stock standards was similar to the one developed in our previous work[28], and contained 2% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, and 0.95% (w/v) high-purity NaCl (Aldrich, 99.999% trace metals basis) as a matrix-matching component. The primary intermediate standard solution was comprised of 1.00 mg L^{-1} of Co, Cd, Sb, Hg, Tl, and U, and 10.0 mg L^{-1} of Cr, Mn, As, Se, Sr, Mo, and Pb. This primary standard solution was stored in a Teflon volumetric flask in a 4°C refrigerator, and was stable up to a month. A series of secondary intermediate standard solutions were prepared by diluting the primary standard with the synthetic-matrix solution, which were stable up to a week when stored in pre-cleaned polypropylene centrifuge tubes at 4°C.

The sample diluent used to prepare working calibration standards and urine specimens was comprised of 1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, and three ISTDs: Ga, Rh, and Ir. The Ir concentration was as low as 1 μg L^{-1} to minimize the potential interference of $^{191}Ir^{16}O$ on ^{207}Pb signals. Triton X-100 (0.05%, w/v) was also added to alleviate possible carryover contaminations between analytical runs and improve nebulization. As a carbon source, three organic solvents, methanol, ethanol, and n-butanol, were examined for their effects on ICP-MS performance.

Working calibration standards were prepared daily by mixing 1 part of the secondary intermediate standards with 9 parts of the sample diluent. Final levels of the analytes in four working calibration standards ranged from 0.01 to 1 μg L^{-1} for Co, Cd, Sb, Hg, Tl, and U, and

from 0.1 to 10 μg L^{-1} for Cr, Mn, As, Se, Sr, Mo, and Pb. The calibration blank was prepared by diluting the synthetic-matrix solution 1:10 with the sample diluent.

Urine analysis – During an analytical run, a urine specimen and intermediate calibration standard, after diluted 1:10 with the sample diluent, was rapidly drawn into the sample loop using a high speed ISIS pump while carrier solution, identical to a calibration blank, was directed to the ICP-MS nebulizer. The minimum urine volume required for a single analysis was 200 µL, which can be mixed with 1.80 mL of the sample diluent. When the ISIS valve was switched from loading to injecting mode for signal acquisition, the carrier solution pushed the sample out of the sample loop to the nebulizer. Simultaneously, the sample probe moved to a rinse port where the DI water was supplied to flush the autosampler probe and tubing with the high-speed ISIS pump. Compared to the conventional sample introduction with peristaltic pump tubing, the use of the ISIS can considerably reduce not only run time, but also carryover contamination by minimizing amounts of sample reaching the nebulizer. Details on the ISIS configuration are described in **Error! Reference source not found.** 1.

Results and Discussion

Cell gas flow rate – Helium was used in this study as a collision gas to eliminate or reduce a variety of polyatomic interferences affecting accuracy during ICP-MS analysis. In a collision cell, polyatomic ions collide with a non-reactive collision gas more frequently than analyte ions because of their relatively larger size, lose more energy, and are then excluded from the ion beam by a kinetic energy barrier. In general, effectiveness of removing polyatomic interferences is proportional to the He flow rate through the collision cell. An analyte-to-background signal ratio at a relevant mass-to-charge ratio (m/z) can vary substantially depending on the level of polyatomic interferences.

Fig. 1a shows the effect of He flow rate on background signals for the analytes of interest using a blank solution containing 0.095% NaCl, 0.1% H_4EDTA, 1.1% NH_4OH, 2.7% n-butanol, and 0.045% Triton X-100. The blank solution was directly aspirated into the ICP-MS at a rate of 0.3 mL min^{-1} and background signals were counted with an integration time of 1 sec. Background signals were greatest when the He flow rate was < 1 mL min^{-1} and decreased dramatically with

increasing He flow rate. With a He flow rate of 1.0 mL min^{-1}, m/z 52 had the highest interference signal (3.1×10^6 cps), which was followed by m/z 78 (4100 cps), m/z 75 (2100 cps), and m/z 55 (1900 cps). However, the background intensities at the m/z 52, 55, 75, and 78 were as low as 390, 23, 1, and 3 cps, respectively, with a He flow rate of 5.5 mL min^{-1}. This demonstrated that a majority of polyatomic interferences such as $^{40}Ar^{12}C$, $^{37}Cl^{18}O$, $^{40}Ar^{35}Cl$, and $^{40}Ar^{38}Ar$ that were spectrally overlapping ^{52}Cr, ^{55}Mn, ^{75}As, and ^{78}Se, respectively, were eliminated. The elevated background signals at m/z 88 (> 1000 cps), which was clearly observed with a He flow rate between 0.0 and 4.0 mL min^{-1}, was proved to be Sr impurities originating from NaCl added to the synthetic-matrix solution.

Fig. 1b illustrates the effect of He flow rate on analyte-to-background signal ratios. Analyte signals were measured with an integration time of 1 sec using a standard solution spiked with 0.1 μg L^{-1} of Co, Cd, Sb, Hg, Tl, and U, and 1 μg L^{-1} of Cr, Mn, As, Se, Sr, Mo, and Pb, and divided by background signals measured for Fig. 1a. Analyte-to-background signal ratios were enhanced for all the analytes as the He flow rate increased, with the greatest improvement observed from m/z 75, followed by m/z 78 and 52. For example, when the He flow rate increased from 0.0 to 5.5 mL min^{-1}, the analyte-to-background signal ratios for m/z 75, 52, and 78 increased by factors of 290, 29, and 25, respectively.

It should be noted that analytical precision, expressed as the relative standard deviation (RSD) of ten replicate measurements of analyte signals in the standard solution, was deteriorated by a high He flow rate (Fig. 1c). For all analytes, RSDs were consistently low (< 6%) as the He flow rate increased from 0.0 to 5.0 mL min^{-1}, but gradually increased for several analytes after 5.0 mL min^{-1}. Especially, RSDs of Se and Cd increased to > 10% when the He flow rate was ≥ 6.0 and ≥ 6.5 mL min^{-1}, respectively. Based on the observations illustrated in Fig. 1a – 1c, 5.5 mL min^{-1} was selected as an optimal He flow rate to achieve low background signals, sufficient analyte-to-background signal ratios, and acceptable analytical precisions across the entire mass range of the analytes of interest.

Carbon-containing organic solvents – In ICP-MS analysis, signal intensities of elements with high first ionization potentials such as As, Se, and Hg can be considerably improved via charge

transfer from carbon ions to the analyte atoms.[29] Direct addition of methane to the nebulizer or spray chamber demonstrated a significant improvement in the instrumental sensitivity for those elements in several studies.[29-31] Alternatively, organic solvents such as methanol, ethanol, and butanol have been widely used in ICP-MS analysis[32-34] as such solvents are known to increase solubility of organic components in bio-specimens.[35] For this study, three commonly used organic solvents, methanol, ethanol (200 proof), and n-butanol, were investigated for their effects on the analytical performance of the ICP-MS (Fig. 2). All solutions prepared for these tests contained 0.095% (w/v) NaCl, 1.1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, 0.045% (w/v) Triton X-100, 0.25 µg L^{-1} of Co, Cd, Sb, Hg, Tl, and U, and 2.5 µg L^{-1} of Cr, Mn, As, Se, Sr, Mo, and Pb. Both carbon enhancing and suppressing effects were clearly observed for all the analytes as well as ISTDs in the presence of organic solvents. For the three solvents tested, the relative sensitivity improvement, compared to counts measured without a carbon-containing solvent, was greatest for Se (360 – 500%) and As (360 – 440%), followed by Sb (160 – 180%) and Mn (120 – 140%). These results are consistent with previous studies that reported the carbon enhancement effect on As and Se signals by organic solvents in ICP-MS analysis.[33, 36, 37] Such signal enhancement was minimal for Tl, Pb, and U (110 – 120%). Considering effects of individual organic solvents, the maximum signal intensities for As and Se were considerably higher with n-butanol (440 and 500%, respectively), compared to ethanol (390 and 420%, respectively), and methanol (360 and 360%, respectively). Similarly, the signal intensity for Sb was higher with ethanol and n-butanol (180%) than methanol (160%). In general, addition of a volatile organic solvent decreases surface tension of solutions[33] and increases evaporation of droplets in the spray chamber[10]. Such changes in solution properties are known to favor smaller droplets, possibly resulting in signal enhancement of analytes.[10, 38] However, given a greatly lower vapor pressure of n-butanol (0.80 kPa at 20 °C) than that of methanol (13 kPa at 20 °C) and ethanol (6.0 kPa at 20 °C), and with comparable surface tensions of these solvents (22 – 24 mN m^{-1} at 20 °C), effects of these parameters on analyte signal intensities appeared to be minimal.

Interestingly, maximum signal enhancement for all the analytes was achieved with n-butanol at a much lower concentration than methanol and ethanol. For example, Se and As reached their maximum intensity with 8% (v/v) of methanol, 6% of ethanol, and 3% (v/v) of n-butanol. Molar concentration of carbon in 3.0% (v/v) of n-butanol is 1.3 mole L^{-1}, which is equivalent to 5.3%

(v/v) of methanol and 3.8% (v/v) of ethanol, indicating that n-butanol, at the same carbon molar level, resulted in greater signal enhancements than methanol or ethanol. Similarly, 2% (v/v) of n-butanol was enough to obtain a maximum signal intensity for Sb, but methanol and ethanol needed to be as high as 4% to achieve maximum enhancement. The rest of the analytes had a maximum intensity with 2.0 – 3.0% of methanol and ethanol, or 1.0 – 2.0% of n-butanol.

Further tests were also conducted to determine whether the added organic solvent causes any adverse polyatomic interferences on analyte background signals. Preliminary experiments showed that ^{52}Cr, mainly an overlap of $^{40}Ar^{12}C$, was the only analyte impacted by an elevated level of these high-purity solvents. Fig. 3 illustrates ^{52}Cr counts from a calibration blank, i.e., 0.095% (w/v) NaCl, 0.1% (w/v) H_4EDTA, 1.1% (w/v) NH_4OH, and 0.045% (w/v) Triton X-100, with various amounts of methanol, ethanol, and n-butanol. When the solvent concentration was < ~6% (v/v), ^{52}Cr intensity was greatest with n-butanol, in the order of butanol > ethanol > methanol. When the solvent concentration was > 7% (v/v), ^{52}Cr intensity increased with increasing methanol and ethanol contents, but decreased with increasing n-butanol levels. For the solvent concentrations that maximized the signal intensities of As and Se in Fig. 2, background signals of ^{52}Cr were 560 cps with 8% of methanol and 6% of ethanol, and 420 cps with 3% n-butanol. Based on these results observed in Fig. 2 and Fig. 3, 3% n-butanol (after dilution with calibration standards urine) would be preferred over 8% of methanol or 6% of ethanol for better sensitivities and lower background signals for the analytes of interest including Cr.

Finally, it should be noted that an optimal solvent concentration needs to be high enough to begin suppressing signals of all the analytes including As and Se, i.e., > 8 % (v/v) for methanol, > 6% (v/v) for ethanol, and > 3% (v/v) for n-butanol. In the presence of this "excess" amount of carbon, the addition of naturally occurring carbon in urine will suppress both analytes and ISTDs in the similar way as organic solvent does, leading to the appropriate internal standardization of analytes. In other words, if the solvent concentration is lower than these optimal concentrations, the urinary carbon may contribute to an increase in As, Se, Sb, or Hg signals but suppress signals of the ISTDs, causing an undesirable bias in analytical results for these analytes. When urine specimens are typically diluted 1:10 with the diluent in this study, a recommended optimal

concentration of n-butanol would be 4% (v/v) in the sample diluent, i.e., 3.6% after dilution with urine, along with 1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, and ISTDs.

Memory effect of Hg – Determination of Hg by ICP-MS can be affected by its infamous memory effect in the sample introduction system including sample probe, nebulizer, and spray chamber. When a washout time between runs is not long enough, analytical accuracy and precision can deteriorate, or a calibration curve may become non-linear. Various approaches have been evaluated in other studies to minimize the memory effect of Hg in ICP-MS analysis, including addition of HCl,[39, 40] gold,[3] EDTA,[3, 19] dichromate,[39] or compounds containing a sulfhydryl group[41, 42] to sample diluent, washing or carrier solution.

Simple experiments were carried out with a Hg standard solution and urine spiked with Hg to compare the washout efficiency of the basic diluent adopted in this study with a commonly used diluent for ICP-MS analysis, diluted nitric acid spiked with gold (Au), as described in Table 2. A "blank" carrier solution was aspired into the ICP-MS nebulizer while the sample loop was filled with one of the prepared solutions, which was then delivered into the nebulizer using the six-way switching valve on the flow injection ISIS. The six-way valve, 100 sec after injection, was switched back to the loading mode, re-directing the carrier solution to the nebulizer. Intensities of the ^{202}Hg signal were monitored throughout these procedures with an integration time of 1 sec, and average values of 10 runs (or 10 sec) were recorded. These experiments were repeated 3 to 4 times with each solution and average values were plotted with standard deviations in Fig. 4. For both standard and urine, Hg intensities reached a plateau in 20 sec with insignificant differences between basic and acidic diluents after the valve was switched to the injecting mode. For the Hg standard solution, after switching back to the loading mode, the acidic diluent needed 140 sec for the Hg level to decrease from 50 to 0.03 $\mu g\ L^{-1}$ or 3 times the method detection limit (MDL) of Hg (Fig. 4a). When the basic diluent was used for the Hg standard, in contrast, it took only 60 sec for Hg concentration to reach the 3 times the Hg MDL.

Similar experiments were conducted using human urine spiked with 45 $\mu g\ L^{-1}$ of Hg, which was diluted 1:10 with each diluent prior to analysis (Fig. 4b). When the acidic diluent was applied, 220 sec was necessary for the Hg level of the spiked urine to fall from 45 $\mu g\ L^{-1}$ to 3 times the

MDL, which was noticeably longer than the washout time needed for the Hg in the standard solution prepared in acid diluent. The longer washout time may be attributed to loss in effectiveness of Au as a preservative of Hg in the presence of organic compounds in urine.[39] When the basic diluent was used to dilute the spiked urine, the washout time was as short as 60 sec, comparable to what was necessary to washout the Hg standard in the basic diluent. These results clearly demonstrated that the basic diluent was far more effective in reducing the Hg memory effect than the acidic diluent containing 2% of nitric acid and 1 μg L^{-1} of Au. The effectiveness of the basic diluent to remove the Hg memory did not seem to be deteriorated by natural organic materials from urine.

Matrix-matched calibration – In this study, intermediate calibration standards were prepared with a basic solution containing 2% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, and 0.95% (w/v) NaCl to matrix-match urine specimens. Both urine and intermediate calibration standards were diluted 1:10 with a diluent containing 0.1% (w/v) H_4EDTA, 1% (w/v) NH_4OH, 4% (v/v) n-butanol, 0.05% (w/v) Triton X-100, and ISTDs, i.e., Ga, Rh, and Ir, as discussed in the previous section. Relative intensities of the ISTDs observed during a typical analytical batch in relation to those observed from ^{23}Na are illustrated in Fig. 5. The relative intensities of the ISTDs were generally consistent throughout the batch, within 100 ± 10% compared to those measured from the calibration blank and standards regardless of Na counts in urine specimens. If the NaCl level in the calibration blank and standards is too low, ISTD intensities in continuous calibration verification (CCV) and blank (CCB) samples would gradually increase as signal suppressing components in urine slowly accumulates after each injection, and vice versa. Insignificant variability in ISTD recoveries observed from CCVs and CCBs in Fig. 5 suggests that the 0.95% NaCl added to intermediate calibration standards effectively matrix-matched the urine specimens that had great variability in inorganic constituents as well as Na content. Considering the wide range of Na signal intensities found in urine specimens, ranging from ~50% to ~110% compared to those in the calibration standards, the intensities of the three ISTDs "co-varied" within a relatively narrow range despite the variation in their first ionization potentials: 6.00, 7.48, and 9.2 eV for Ga, Rh, and Ir, respectively. Moreover, the RSDs of the signal intensities between the three ISTDs were less than 2% for most specimens, which is critical in order to minimize any internal-standard-dependent biases resulting from choosing an "inappropriate" ISTD.

Mo interferences on Cd signal – In contrast to human blood or serum specimens, Mo concentrations in urine are generally two to three orders of magnitude greater than the typical Cd level of < 0.5 μg L^{-1}.[3, 5-7] Elevated Mo can make it very challenging to quantify urinary Cd concentrations with accuracy due to potential polyatomic overlap of Mo oxides such as $^{98}Mo^{16}O^{+}$ and/or $^{96}Mo^{18}O^{+}$ on $^{114}Cd^{+}$ signals.[43] Cadmium has eight natural isotopes with various relative abundances: ^{106}Cd, 1.25%; ^{108}Cd, 0.89%; ^{110}Cd, 12.49%; ^{111}Cd, 12.80%; ^{112}Cd, 24.13%, ^{113}Cd, 12.22%; ^{114}Cd, 28.73%; ^{116}Cd, 7.49%. Except for ^{111}Cd, there are problematic isobaric interferences on the Cd isotopes such as ^{110}Pd, ^{112}Sn, ^{113}In, ^{114}Sn, etc. In addition, every Cd isotope except for ^{106}Cd has the possibility of spectral interferences from the MoO formation. Such polyatomic interferences can be eliminated by the ICP-MS instrument using dynamic reaction cell technology with oxygen gas,[43, 44] but cannot be fully resolved with a He-mode collision cell. As an alternative, in this study, correction of Mo oxide inferences on Cd was attempted using an empirical equation.

In order to select a Cd isotope with minimal interference from Mo, a series of Mo standard solutions (5, 10, 50 and 100 μg L^{-1}) were prepared with 0.095% (w/v) NaCl, 1.1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, 3.6% (v/v) n-butanol, and 0.045% (w/v) Triton X-100. Count ratios of $^{X+16}$Cd to ^{X}Mo were measured with a He flow rate of 5.5 mL min^{-1} and results are averaged in Table 3. For simplification, it was assumed that ^{16}O (99.76%) is the only isotope associated with MoO formation. Several Cd isotopes were excluded from consideration due to their low abundance (i.e., ^{106}Cd and ^{108}Cd) and potential isobaric interferences from ^{113}In and ^{116}Sn.

Average $^{X+16}$Cd/^{X}Mo count ratios (n = 22) in these four Mo standard solutions were relatively comparable for all Cd isotopes tested, varying from 0.000850 ± 0.000303 for ^{110}Cd/^{94}Mo to 0.00103 ± 0.00018 for ^{114}Cd/^{98}Mo. A low ^{110}Cd/^{94}Mo ratio can be explained by a relatively low abundance of ^{94}Mo (9.25%), suggesting that ^{110}Cd is the isotope with the least MoO interference. However, in the absence of Mo in the solution, the background signal for ^{110}Cd (77 ± 12 cps) was significantly higher than those of ^{111}Cd (1.2 ± 0.6 cps), ^{112}Cd (3.6 ± 1.2 cps), and ^{114}Cd (3.9 ± 1.7 cps), suggesting an unknown polyatomic interference(s) on ^{110}Cd signal. Besides ^{110}Cd, an average background level in the presence of 100 μg L^{-1} of Mo was lowest for ^{111}Cd (540 ± 60

cps) and highest for ^{114}Cd (1010 ± 120 cps). The higher background of ^{114}Cd can be attributed to the relatively high abundance of ^{98}Mo (Table 3). Considering relatively low concentrations of Cd in human urine,[3, 6, 7] ^{111}Cd was selected for this study due to its lower backgrounds than the other isotopes.

Intensities of ^{111}Cd were corrected using the following equation:

$$^{111}Cd_{corr} = {}^{111}Cd - CF \times {}^{95}Mo$$

where CF is an empirical correction factor based on an average count ratio of ^{111}Cd to ^{95}Mo measured under a He flow rate of 5.5 mL min^{-1} from the four Mo standard solutions (5, 10, 50, and 100 µg L^{-1}) prepared with 0.095% (w/v) NaCl, 1.1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, 3.6% (v/v) n-butanol, and 0.045% (w/v) Triton X-100. Due to the temporal variability observed in the correction factor with a range from 0.000721 to 0.00127 (n = 22), correction factors were determined before and after every analytical batch, and then an average of two values was used for the correction equation for that batch. The relative percent differences (RPDs) between correction factors determined before and after an analytical batch were typically < 15% (n = 9).

Fig. 6 compares Cd recoveries with and without Mo-interference correction for selected standard reference materials (SRM) from the National Institute of Science and Technology (NIST 2668) and Sero (Seronorm™ urine), as well as proficiency testing (PT) urine specimens from the institut national de santé publique du Québec (INSPQ 1509, 1510, 1515, and 1516). Results clearly demonstrated that corrected Cd concentrations were closer to certified/reference values than uncorrected concentrations with the exception of Seronorm L1. A low recovery (75%) after the empirical correction for Seronorn L1 might be attributed to the low target level (0.20 µg L^{-1}) with a relatively large uncertainty (0.04 µg L^{-1}). The difference between the corrected and uncorrected Cd values was relatively small (RPD < 10%) in Seronorm L2, NIST 2668 L1, INSPQ 1515, 1516, and 1510 where the Mo to Cd concentration (in µg L^{-1}) ratio was < 50. However, the RPD was > 20% when the Mo to Cd concentration ratio was > 100 as observed in NIST 2668 L2, Seronorm L1, and INSPQ 1509. Considering Mo/Cd concentration ratios are typically far greater than 100 in natural human urine,[3, 5-7] this empirical approach can minimize the bias in analytical results from the Mo interference.

Method Validation – The method detection limits (MDLs) were experimentally estimated for the thirteen analytes by measuring seven replicates of a human urine specimen with low levels of the analytes of interest and multiplying the standard deviation of these measurements by a factor of 3.143. Method detection limits, based on 1:10 dilutions, were as follows: 0.0410 μg L^{-1} for Cr, 0.0294 μg L^{-1} for Mn, 0.0170 μg L^{-1} for Co, 0.162 μg L^{-1} for As, 1.15 μg L^{-1} for Se, 0.867 μg L^{-1} for Sr, 0.200 μg L^{-1} for Mo, 0.0391 μg L^{-1} for Cd, 0.0101 μg L^{-1} for Sb, 0.0101 μg L^{-1} for Hg, 0.00436 μg L^{-1} for Tl , 0.0168 μg L^{-1} for Pb, and 0.00120 μg L^{-1} for U. It should be noted that the MDLs of As, Se, Sr, and Mo are much higher than instrumental detection capability due to naturally elevated levels of these elements in the urine specimen used for the MDL experiments. In general, these MDLs are not only comparable with or lower than values reported by other recent studies conducted with a quadrupole-based ICP-MS,[3, 6, 7, 14, 15, 18] but also low enough to determine typical levels of these elements in a normal population.

Fig. 7 presents the analytical results from repeated analyses of SRM and PT specimens. Average recoveries were 100 ± 10% for 90% of certified/reference values. Lower (< 90%) recoveries were found from Mn, Cd and U in Seronorm L1, Co in NIST 2668, Cd and Se in INSPQ 1509, and Hg in NIST 3668. The average recovery of U from Seronorm L2 was 159 ± 55% (0.0366 ± 0.0127 μg L^{-1}, n = 38), compared to the reference value (0.023 μg L^{-1}). This large variability was attributed to substantial heterogeneity between six randomly selected vials from a single lot. Although intra-vial (within a vial) variabilities as RSDs were lower than 8%, an average U concentration in each vial varied from 0.00229 to 0.00519 μg L^{-1}: 0.0229 ± 0.0017 (n = 7), 0.0243 ± 0.0017 (n = 7), 0.0286 ± 0.0023 (n = 6), 0.0470 ± 0.0015 (n = 7), 0.0500 ± 0.0029 (n = 5), and 0.00519 ± 0.0025 μg L^{-1} (n = 6). This inter-vial (between vials) variability was probably caused by incomplete homogenization of the elemental components during manufacturing. The elevated recovery of Hg in Seronorm L1 (0.0545 ± 0.0079 μg L^{-1}, 152±22%) did not appear to be associated with such inter-vial variability observed from U. The certified value (0.036 μg L^{-1}) is merely as low as ~3 times MDL (0.0101 μg L^{-1}) and our results were still within the acceptable range (0.015 – 0.057 μg L^{-1}).

Long-term stability of this analytical method was evaluated using three levels of internal quality control (QC) materials that were prepared with pooled human urine specimens spiked with the

analytes of interest. The internal QC materials were analyzed at both the beginning and end of each analytical batch. The daily reproducibility or relative percent difference calculated from a pair of results was 2.4 – 3.1%, 1.5 – 2.0%, and 1.6 – 2.2% (95% confidence interval) for low, medium, and high-level QC materials, respectively. Long-term precision, as RSD, was 3.2 – 3.9% (95% confidence interval) for all the analytes except for Hg (8.0%) over a period of 60 days.

Conclusions

We reported an ICP-MS method for determination of thirteen trace metals and metalloids, Cr, Mn, Co, As, Se, Sr, Mo, Cd, Sb, Hg, Tl, Pb, and U, in human urine. In contrast to a majority of previous ICP-MS methods for human urine analysis, this method was developed to simultaneously determine all the analytes, including Hg, using a single analytical condition. Effects of cell gas flow rate and diluent composition were critically evaluated to minimize polyatomic interferences and maximize analyte signal intensities. Polyatomic Mo oxide interferences were empirically removed from Cd signals, considerably improving Cd recoveries from SRM and PT specimens. Sodium chloride added to calibration standards successfully matched the signal suppression of analytes in urine specimens, stabilizing internal standard signals during analytical batches regardless of the great variability commonly observed from organic and inorganic constituents in urine. Additional advantages of this method include excellent washout efficiency for Hg. The present method is ideal for analytical laboratories which require high-throughput analysis (< 3 min/sample) with limited urine volumes (200 uL). With the relatively simple analytical conditions of this method, it can still produce analytical results with superior detection capability, accuracy, and precision.

Live Subject Statement

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C.) 263a) as revised by the Clinical Laboratory Improvements Amendments (CLIA), the California Dept. of Public Health, Environmental Health Laboratory Branch may accept human specimens for the purposes of performing laboratory examinations or procedures (CLIA ID Number 05D0882397, exp. May 2017). The urine specimens used for quality control pools were collected anonymously with informed consent from the donors.

Acknowledgements

The authors thank Drs. Jed Waldman, Jianwen She, and anonymous reviewers for their important contributions to the manuscript, Josephine DeGuzman, Jun-Qiang Zhou, and Jeffrey Aduviso for laboratory assistance and sample analysis. This work was supported by Cooperative Agreement no. 5U88EH001148 from the Centers for Disease Control and Prevention and does not necessarily reflect the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

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Table 1 Instrumental settings and isotopes monitored.

Parameter	Setting	
ICP-MS		
RF power	1550	W
Plasma gas	15.0	L min^{-1}
Nebulizer gas	0.96	L min^{-1}
Dilution gas	0.17	L min^{-1}
Carrier solution pump	0.3	mL min^{-1} (0.1 rps)
$^{140}Ce^{16}O^{+}/^{140}Ce^{+}$ (no gas)	< 1.5	%
$^{140}Ce^{2+}/^{140}Ce^{+}$ (no gas)	< 1.5	%
ISIS		
Sample loop internal volume	780	µL
Sample load time	14	sec
Sample load peristaltic pump	13	mL min^{-1} (1.0 rps)
Read-delay time	25	sec
Rinse time between samples	60	sec
Rinse peristaltic pump	13	mL min^{-1} (1.0 rps)
Isotope and integration time		
^{52}Cr, ^{78}Se, ^{111}Cd, and ^{202}Hg	2.0	sec
^{55}Mn, ^{59}Co, and ^{75}As	1.5	sec
^{88}Sr, ^{95}Mo, ^{121}Sb, ^{205}Tl, ^{206}Pb, ^{207}Pb, ^{208}Pb, and ^{238}U	1.0	sec
^{71}Ga, ^{103}Rh, and ^{193}Ir (internal standards)	0.7	sec

Total Pb = $^{206}Pb + ^{207}Pb + ^{208}Pb$
Total acquisition time = Σ integration time (22.6 sec) × 3 replicates = 68 sec
Total run time = sample load + read-delay + acquisition + rinse = 167 sec

Table 2 Sample preparation for Hg memory effect tests

	Basic Solution	Acidic Solution
Synthetic matrix	2% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, and 0.95% (w/v) NaCl	2% (v/v) HNO_3, 1 mg Au L^{-1}, and 0.95% (w/v) NaCl
Intermediate standard	2% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, 0.95% (w/v) NaCl, and 50 µg Hg L^{-1}	2% HNO_3, 1 mg Au L^{-1}, 0.95% NaCl, and 50 µg Hg L^{-1}
Diluent	4% (v/v) n-butanol, 1% (w/v) NH_4OH, 0.1% (w/v) H_4EDTA, 0.05% (w/v) Triton X-100, and ISTDs	4% (v/v) n-butanol, 2% (w/v) HNO_3, 1 mg Au L^{-1}, 0.05% (w/v) Triton X-100, and ISTDs
Working solutions		
Carrier solution	1 part synthetic matrix	+ 9 parts diluent
Standard (Fig. 4a)	1 part intermediate standard (50 µg L^{-1})	+ 9 parts diluent
Urine (Fig. 4b)	1 part spiked urine (45 µg L^{-1})	+ 9 parts diluent

Table 3 Mo interferences on Cd signals (n = 22)

Cd isotope (% abundance)	Mo isotope (% abundance)	Average Cd/Mo count ratio	Cd in blank (cps)	Cd in 100 μg L^{-1} Mo (cps)
^{110}Cd (12.49%)	^{94}Mo (9.25%)	0.000850 ± 0.000303	77 ± 12	370 ± 40
^{111}Cd (12.80%)	^{95}Mo (15.92%)	0.000958 ± 0.000175	1.2 ± 0.6	540 ± 60
^{112}Cd (24.13%)	^{96}Mo (16.68%)	0.00100 ± 0.00017	3.6 ± 1.2	610 ± 80
^{114}Cd (28.73%)	^{98}Mo (24.13%)	0.00103 ± 0.00018	3.9 ± 1.7	1010 ± 120

Fig. 1 Effects of helium flow rate through the collision cell on (a) background signals, (b) analyte-to-background signal ratios, and (c) precisions as RSD for analytes of interest

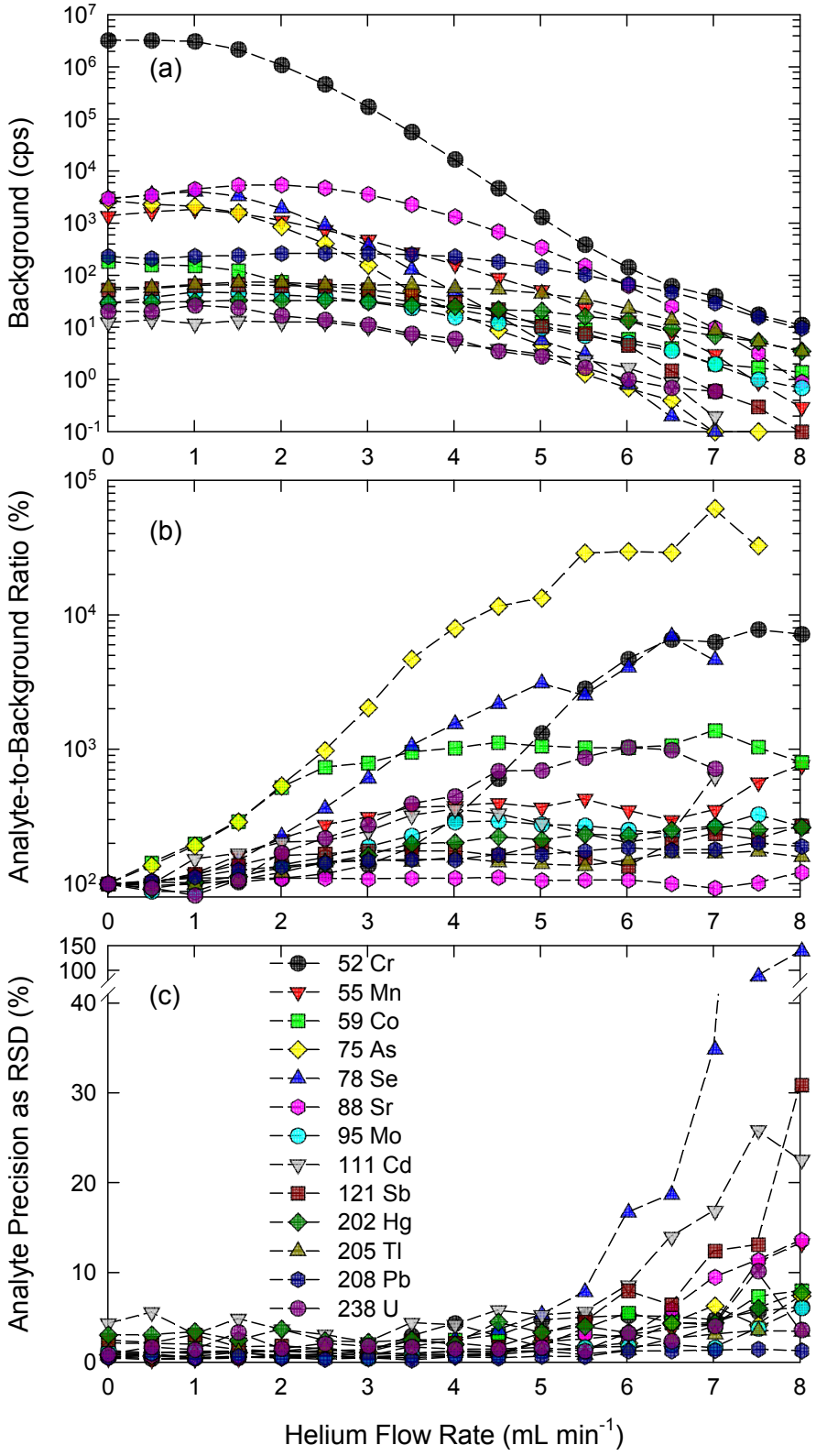

Fig. 2 Effects of (a) methanol, (b) ethanol, and (c) n-butanol on analyte signal intensities

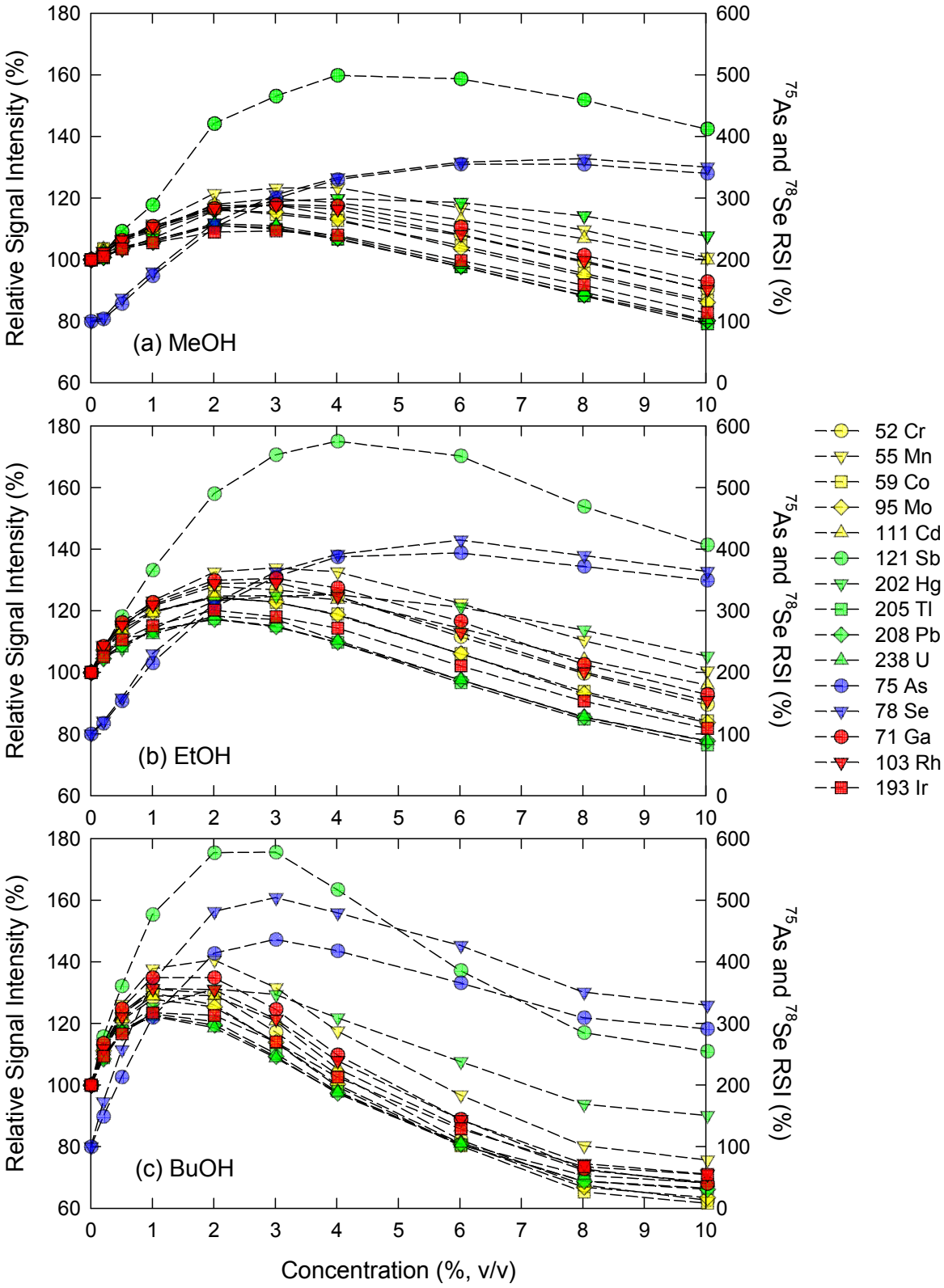

Fig. 3 Background signals of ^{52}Cr in calibration blank (0.095% NaCl, 0.1% H_4EDTA, 1% NH_4OH, 0.045% Triton X-100) in the presence of 5.5 mL min^{-1} of helium as collision gas (error bars are standard deviation from 10 runs)

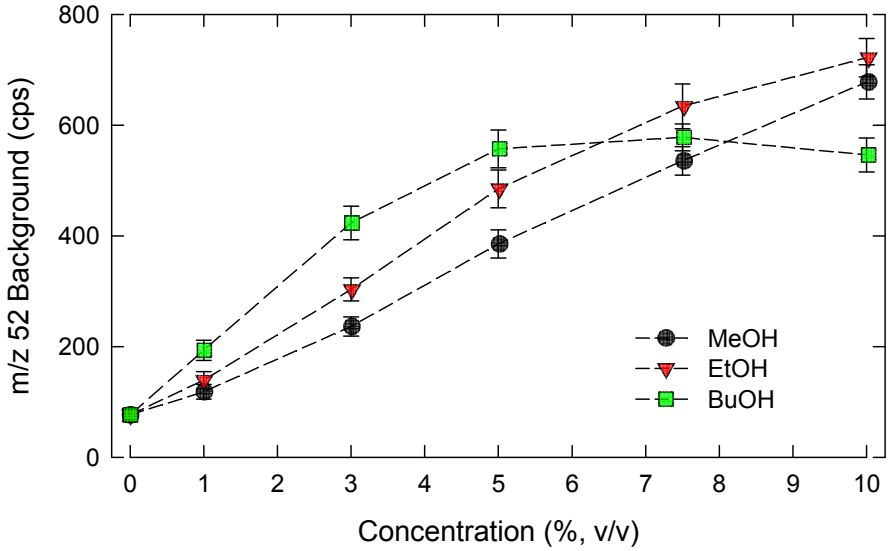

Fig. 4 Comparison of washout efficiency between the basic and acidic diluents on the Hg memory effect from (a) standard and (b) urine. The ISIS valve was switched from the loading to the injecting mode at -100 sec and then back to the loading mode at 0 sec.

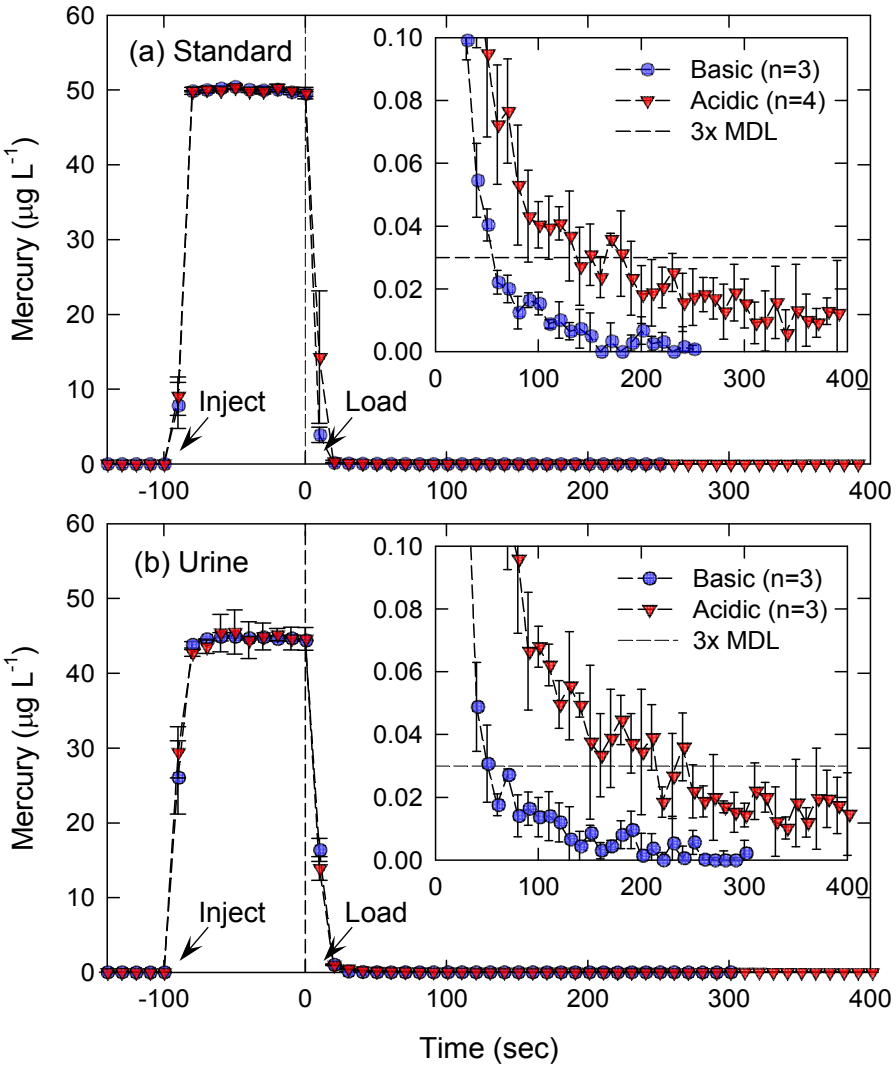

Fig. 5 Short-term stability of internal standards during an analytical batch

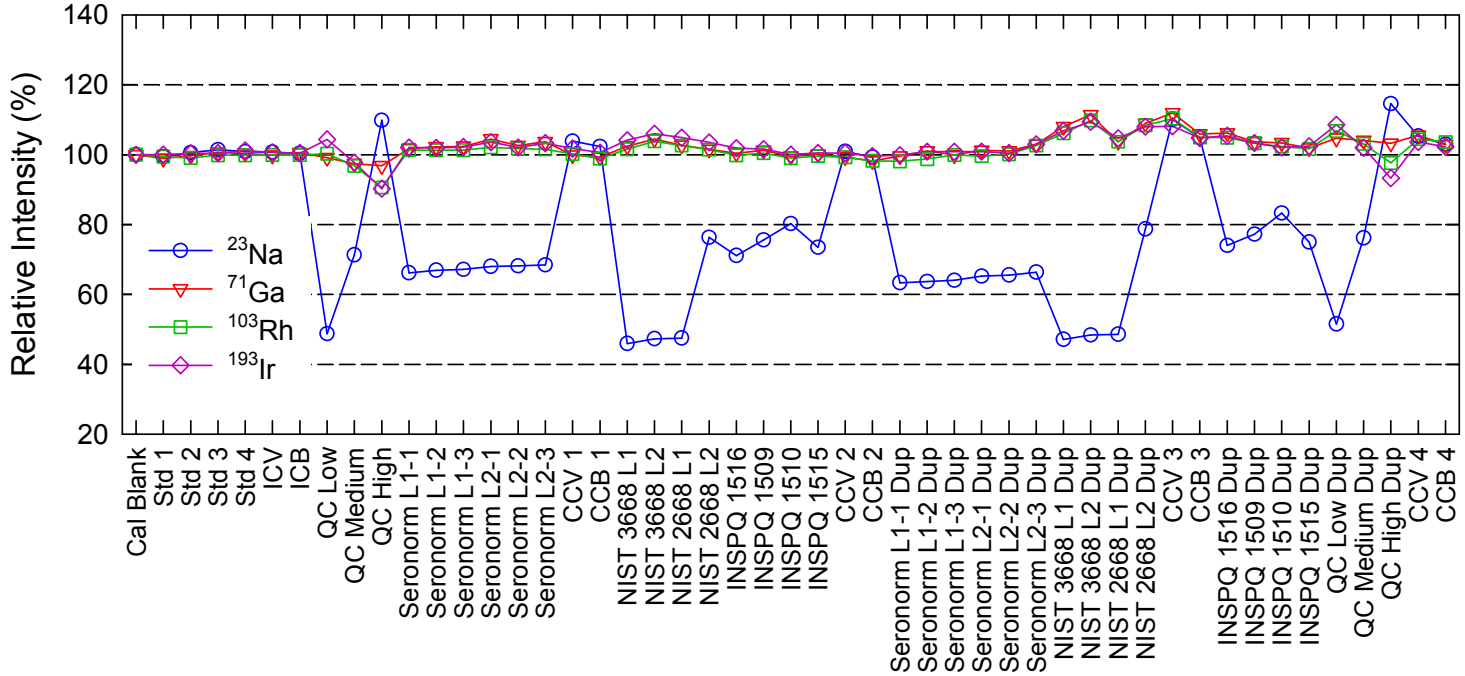

Fig. 6 Comparison of Cd values with and without Mo-interference correction. The numbers within parentheses on the x-axis are certified or reference values of Cd in a unit of μg L^{-1}. Error bars represent standard deviations.

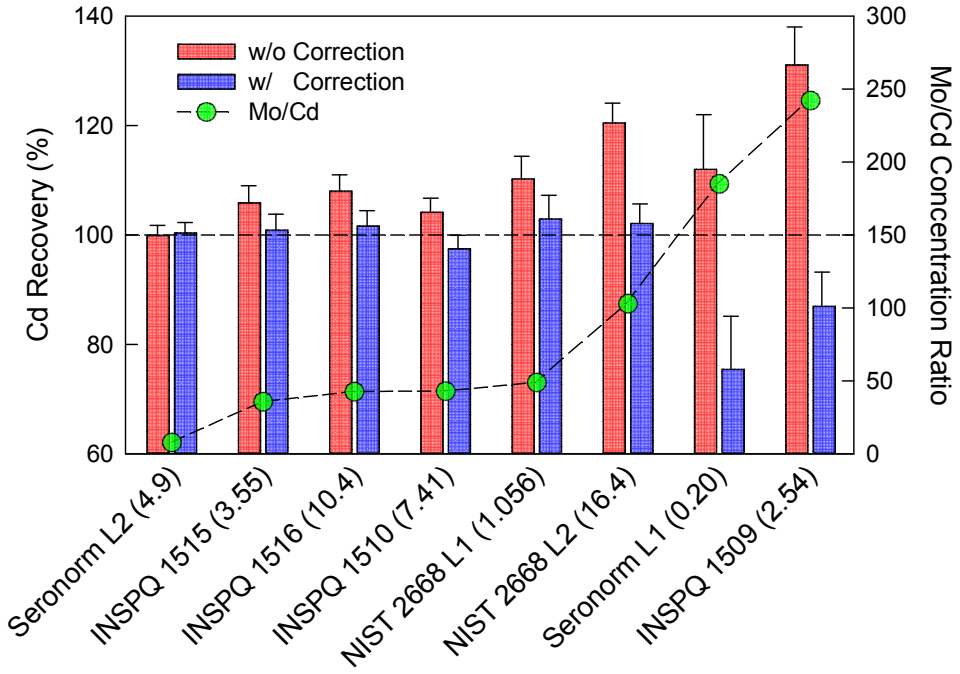

Fig. 7 Average recoveries of thirteen analytes from SRM and PT specimens