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Determination of Ultra-trace Elements in Human Plasma or Serum by ICP-MS using Sodium in the Presence of Carbon as a Single Calibration Matrix-Match Component

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Abstract

A sensitive and high-throughput method was developed for ultra-trace analyses of Mn, As, Cd, W, Hg, Pb and U in plasma or serum specimens using inductively coupled plasma mass spectrometry (ICP-MS) equipped with a He mode collision cell. Calibration standards were prepared in basic solution with NaCl and n-butanol (present in the diluent) as Na and C sources, respectively. The remaining components of the diluent were, NH_4OH, H_4EDTA, Triton X-100 and internal standards (Ga, Rh, Re and Ir). Both calibration standards and plasma specimens were diluted 1:10 and directly injected, using an integrated sample introduction system (ISIS), to the ICP-MS bypassing the time consuming and contamination prone chemical/heat digestion steps used elsewhere. The addition of 2% n-butanol to the calibration standards caused a substantial signal enhancement - as much as 450% for As and 120-130% for the other elements - due to the charge transfer from carbon ions (C^+) to analytes in the instrument plasma. Further increase in n-butanol concentration steadily decreased elemental signal intensities in a very similar way as the addition of NaCl. Both C^+ and Na^+ signal intensities in the instrument plasma were at a similar level and their suppressing effect on analytes and internal standards seemed to be interchangeable. Therefore, a thermodynamic approach where one or more ions at higher concentration can influence ionization of other elements present in the instrument plasma at much lower concentrations, which would describe the observed phenomena. These findings were helpful to determine the optimal concentration of 1% NaCl in the intermediate calibration standards and 4% of n-butanol in the diluent to matrix-match the suppressing effect of inorganic and bio-organic components of plasma specimens.

Introduction

The liquid portion of blood is a complex mixture of 95% water and 5% suspended or dissolved biomolecules, nutrients, physiological waste products and inorganic ions. The remaining inorganic components, such as sodium (144 mM), chlorine (110 mM), bicarbonate/carbon dioxide (25 mM), iron (9 mM), oxygen (6 mM), potassium (4.5 mM), calcium (2.5 mM), phosphorus/phosphate and sulfur/sulfide (~1 mM) and magnesium (800 µM), constitute about 1% of the total mass of serum.[1] Depending on preparation methods, the final product from the separation of the cellular portion of blood can be serum or plasma. Plasma is slightly more viscous than serum, as it contains anticoagulants such as fibrinogen, prothrombin and other clotting proteins that were removed from serum.[1] Due to its homeostatic nature, ionic composition of serum or plasma in healthy humans is similar and relatively stable. A host of essential elements such as Mn, although present at trace or ultra-trace levels, play a vital role in maintaining many important functions in the human body. Toxic elements such as Hg, Cd and As have no physiological function and pose a potential human health risk through acute or long term exposure. Precise and accurate determination of trace elements in plasma or serum is essential for human biomonitoring studies, which allows us to compare concentration levels among different study populations and correlate health effects with exposures to various elements.

The most frequently used analytical technique capable of analyzing samples with such complex matrices for (quasi) simultaneous determination of multiple metals is inductively coupled plasma mass spectrometry (ICP-MS). Plasma or serum specimens can be analyzed using a “dilute and shoot” approach, where a specimen is diluted with a diluent and injected directly into the instrument. Commonly used diluents include: deionized water,[2-4] diluted HCl,[5] diluted HNO_3,[6] a solution of acetic acid and Triton X-100,[7] butanol, nitric acid and Triton X-100,[8, 9] ammonia, EDTA and Triton X-100,[10] or butanol, ammonia, EDTA and Triton X-100.[11] Alternatively, serum samples can be thermally digested in HNO_3,[12] a mixture of HNO_3 and H_2O_2,[13-17] or a mixture of HNO_3 and $HClO_4$[18]. Enzymatic digestion using non-specific protease enzymes such as pronase [19],chemical digestion using tetramethylammonium hydroxide [20] and pretreatment with formic acid [21] were also reported. Subsequently, digestates are usually diluted with high purity water or diluted acid and injected into the instrument.

Regardless of preparation methods, the solutions will always have significant amounts of organic and inorganic components that are responsible for non-spectroscopic interferences, e.g., matrix effects (ME). During the last 30 years, numerous studies have investigated effects of the ME on accuracy and precision of analytical determination of multiple metals by ICP-MS.[22-42] In general, the ME can be categorized as enhancing, suppressing or having no

influence on analyte signal intensities.[43] Signal enhancement is usually observed when organic substances are present in the sample solution, [22, 23, 41, 42] and the addition of C originating from moderate amounts of methanol, ethanol, or mannitol increases signals for Ge, As and Se.[23, 29] The addition of ethanol, propanol, butanol, acetonitrile, ammonium acetate or glucose increases signals for Ga, As, Cd, In, Pb, Zn and Se to various degrees.[28] This signal enhancement by addition of C-containing solvents and other organic compounds is mainly attributed to the charge transfer from C^+ ions to those elements with a first ionization potential (FIP) of 9 - 11 eV.[22, 41, 42, 44] The elemental signal intensities are, however, not always proportional to the concentration of organics. Signal suppressions were reported at higher amounts of acetone and methanol in solution.[23] For Ge, As or Se, the presence of concomitant elements such as Cl and S were found to induce an enhancement effect, whereas N and P did not show any significant effect.[29] A noticeable signal increase was observed for Ge, As and Se (few fold) in 1% HCl or H_2SO_4, but not in HNO_3 solution at the same concentration.[28]

Signal suppression is mostly associated with the presence of concomitants including easily ionizable elements (EIE) such as K, Na, Cs, Mg, Ca and Si.[25, 31, 39] The signal suppression is explained by any changes in ion-atom equilibrium in the instrument plasma,[39] or the space charge effect taking place beyond the cones. The space charge effect appears to increase with increasing matrix element mass and decrease with increasing analyte mass.[25, 31] Presence of elevated levels of the EIE in plasma/serum specimens would lead to a potential bias in analytical results if external calibration standards are prepared in water for direct analysis[9, 40, 45] The analytical bias can be minimized if matrix-match is accomplished between calibration standards and sample solutions to compensate for their physicochemical characteristics. As a simple way to achieve a matrix-match, serum addition to calibration standards was proposed.[2, 3, 5, 46] The addition of serum to the calibration blank and standards, however, may possibly result in substantial decreases in accuracy and precision of the analysis if these analytes are also present in the added serum.

Modification of standard solutions by adding a mixed salt solution that contained (per liter) 0.48 g of KH_2PO_4, 0.28 g of $CaCl_2$, 2.90 g of NaCl, 0.07 g of K_2SO_4 and 8.1 g of EDTA (disodium salt) to account for ionic interferences present in plasma was also proposed.[47] In a different approach, 10% v/v CH_3COOH solution was used for matrix-match and also as a C source.[7] Partial elimination of the plasma matrix by chemical or enzymatic digestion (assuming that the final concentration of the enzyme used is negligible) will still leave behind inorganic components in the plasma solution. Both digestion methods would substantially increase sample preparation time, yet these approaches seems to be the latest method of choice for biomonitoring and

other research projects.[13-18] As another alternative, standard addition methods, where known amounts of target analytes are added to subsamples and the unknown analyte concentrations are determined from resulting plots, has also been used.[35, 43] This standard addition method, however, is time consuming because of the need to perform a full calibration for each sample.

All things considered, the most attractive approach to the plasma analysis for ultra-trace metals is the "dilute and shoot" method. Internal standards (ISTDs) should be used to counter the ME and related instrument drift.[2, 3, 46, 48] However, selection of a "good" ISTD for any given analyte in different matrices is often difficult.[49, 50] The best approach would be to select an ISTD for each individual analyte by matching the FIP and atomic mass.[49] Although a given ISTD can be an acceptable match for more than one analyte, multiple ISTDs would still be required for multi-metal analyses.[35]

Previous studies suggested a relatively simple way to minimize the ME for determination of ultra-trace elements in human blood; a mixture of NaCl and $CaCl_2$ was added as a "synthetic matrix" to calibration standards.[40, 51] Our recent study[49] demonstrated that under such matrix-match conditions for blood specimens and calibration standards, any ISTD (Ge, Rh, Re, Ir and Tl) could be selected for any analyte (As, Cd, Hg, Mn, Pb and U) regardless of FIP or atomic mass (i.e., ^{74}Ge was an acceptable ISTD for ^{238}U or ^{205}Tl for ^{55}Mn, etc.). In the present study, a similar matrix-match approach was investigated by adding NaCl as a single calibration matrix-match component to the external calibration standards to compensate for the ionization suppression of the seven target analytes, Mn, As, Cd, W, Hg, Pb and U, and four ISTDs, Ga, Rh, Re and Ir. An optimal concentration was suggested for NaCl in the standard solutions and n-butanol in the sample diluent. In addition, the enhancement and suppression of elemental signals in the instrument plasma were discussed and their effects on analytical results were demonstrated.

Experimental

Instrumentation

An Agilent 7700x quadrupole ICP-MS was used for this study with a He mode collision cell and an integrated sample introduction system with discrete sampling (ISIS-DS or "ISIS") as a flow injection system (Agilent Technologies Inc., Santa Clara, CA, USA). The ICP-MS was operated with platinum sampler and skimmer cones, a MicroMist glass concentric nebulizer and a chilled quartz Scott-type spray chamber (both from Agilent Technologies, Inc.). The Agilent 7700x was interfaced to a CETAC ASX 500 series (Omaha, NE, USA) autosampler that was placed inside an enclosure CETAC ENC 500

(Omaha, NE, USA). The ISIS loop was made of polytetrafluoroethylene (Cole Palmer, Vernon Hills, IL, USA) to minimize carryover of sample components. The instrument settings and parameters are detailed in Tables 1 and 2. All other instrument settings were unchanged throughout the study, except for a few parameter adjustments made by the instrument software during auto-tuning.

Table 1 Agilent 7700x operating parameters

Table 2 Spectrum acquisition parameters

The instrument was routinely checked before each analytical run for sensitivity and interference levels ($CeO^+/Ce^+ < 1.5\%$ and $Ce^{++}/C^+ < 1.5\%$). The analytical run procedure was initiated if all instrumental parameters were within specified ranges. A He flow rate of 4.5 mL min^{-1} in the collision cell was selected to achieve sufficient sample to noise intensity ratios across the mass range of analytes. A typical background intensity on the ^{75}As signal (i.e., the overlap of $^{40}Ar^{35}Cl$ associated with elevated Cl level present in the carrier solution), was approximately 10 cps at this He flow rate. Another possible interference, $^{191}Ir^{16}O^+$ for ^{207}Pb, was minimized (~100 cps) by lowering the Ir concentration to 1 µg L^{-1}.

Calculations of the analytical results for each ISTD were completed using MassHunter v. B.01.01 software. Statistical calculations were performed using Microsoft Excel.

Reagents and solutions

Type 1 deionized (DI) water (≥18.2 MΩ·cm resistivity) produced from a Millipore Milli-Q water purification system (Dubuque, IA, USA) was used for preparation of all aqueous solutions. Stock standard solutions of As, Cd, Mn, Pb, Hg, W and U, each at a concentration of 1,000 mg L^{-1}, and Ga, Rh, Re and Ir (ISTDs), each at a concentration of 1,000 mg L^{-1}, were obtained from SPEX CertiPrep (Metuchen, NJ, USA). A second source of a custom standard solution containing As, Cd, Mn and Pb at 1,000 mg L^{-1} each, and Hg and U at 100 mg L^{-1} each, was prepared by Inorganic Ventures (Christiansburg, VA, USA). All standards were traceable to the National Institute of Science and Technology (NIST, Gaithersburg, MD, USA). High-purity sodium chloride, Triton X-100 and H_4EDTA were obtained from Sigma-Aldrich (Milwaukee, WI, USA), and ammonium hydroxide and n-butanol from Fisher Scientific (Pittsburgh, PA, USA). All the labware were acid cleaned with a mixture of 2% HCl and 1% HNO_3 prior to use and were screened for traces of metal contamination. All the solutions were stored in pre-cleaned Teflon® bottles (Nalgene®, Rochester, NY, USA).

The intermediate standard diluent was an aqueous solution of 2% w/v NH_4OH, 0.25% w/v H_4EDTA and 1% w/v NaCl. The diluent solution for

sample and working standards (sample diluent) was a solution of 2% w/v NH_4OH, 0.25% w/v H_4EDTA, 4% w/v n-butanol, 0.1% w/v Triton X-100 and four ISTDs: Ga, Rh, Re and Ir at 10, 2, 5 and 1 µg L^{-1} respectively. The carrier solution, identical to the calibration blank solution, was made with 1 part of the intermediate standard diluent and 9 parts of the sample diluent, and was used to push the sample from the loop to the nebulizer during signal acquisition. The ISIS washing solution consisted of 2% w/v NH_4OH, 0.1% w/v H_4EDTA and 0.1% w/v Triton X-100. The washing solution provided additional rinse of the autosampler probe and connection tubings as well as the ISIS sample loop and the six-way valve between analytical runs.

Table 3 Metal concentrations in the working calibration standards

The intermediate standards were prepared in two steps:

Step 1: 1.00 mL of As, Cd, Mn and Pb, and 0.100 mL of W, Hg and U were pipetted from the stock standard into a 100 ± 0.16 mL TD/TC at 20℃ Teflon volumetric flask (Nalgene®, Rochester, NY, USA) and adjusted to a final volume of 100 mL with the intermediate standard diluent. Alternatively, 1.00 mL of the six-metal custom stock standard and 0.100 mL of W were used to prepare 100 mL of the solution.

Step 2: Four levels of intermediate standards were prepared by aliquoting varying volumes of the solution from Step 1 into 15 mL polypropylene centrifuge tubes (BD Falcon, Franklin Lakes, NJ, USA), then adjusting each volume to 10.0 mL using the intermediate standard diluent. The first level (blank) was solely the intermediate standard diluent. The intermediate standards were stable for at least 1 week when stored at 4℃. Working standard solutions were prepared by mixing 0.5 mL of each intermediate standard with 4.5 mL of the sample diluent. The final analyte concentrations in the working standards are listed in the Table 3.

Plasma Specimens Analysis

All samples were prepared under a Class II biological safety cabinet. A Digiflex CX (Titertek, Huntsville, AL, USA) was used to dispense 4.5 mL of the sample diluent into pre-cleaned 15 mL polypropylene tubes, followed by 500 µL of plasma specimen or QC sample using a manual Eppendorf pipette (Eppendorf AG, Hamburg, Germany). Any excess plasma or QC sample left outside the tip was carefully wiped away using a standard absorbent wipe from Fisher Scientific (Pittsburgh, PA, USA). The transfer was subsequently completed using a repetitive “pumping” action to assure complete transfer of the material from the pipette tip into solution. A minimum sample volume of 200 µL was required for a single analysis, which was mixed with 1.80 mL of the sample diluent for analysis.

The diluted samples were finally analyzed on the ICP-MS with the ISIS configuration, where the carrier solution is continuously pumped in order to either push the sample out of the sample loop or directly feed the instrument (Fig. 1). During an analytical run the loop was filled with the calibration blank, standard or diluted plasma specimen (Fig. 1A), which was then injected into the instrument when the ISIS valve was switched from loading to injecting mode (Fig.1B).

Once the instrument is equilibrated with the carrier solution, no significant change in ISTD intensities should be observed during the analysis of calibration standards. The calibration was paused and restarted when there were more than 1% changes in the relative signal intensities (RSI) of the ISTDs. All plasma and QC samples were analyzed in duplicate and the average was reported as a final result. Continuing calibration verifications (CCV – identical to second working calibration standard) and continuing calibration blanks (CCB – identical to calibration blank) were inserted after the calibration standards and every ten plasma samples. Carryover of any analyte during analytical runs was negligible.

Figure 1 Schematic diagram of solution flow in the ISIS during a) loading and b) injecting mode. In all experiments, the carrier solution composition was identical to the diluted blank used in the specific run

Method Validation

The method detection limits (MDLs) for each analyte were determined from the standard deviation of seven replicates of human plasma/serum specimens. The MDLs for plasma/serum specimens diluted 1:10 was 0.0155 μg L^{-1} for Mn, 0.00424 μg L^{-1} for As, 0.00237 μg L^{-1} for Cd, 0.00438 μg L^{-1} for W, 0.00676 μg L^{-1} for Hg, 0.00329 μg L^{-1} for Pb and 0.00207 μg L^{-1} for U. These MDLs were comparable as reported in one study [21] or from a fraction to about two orders of magnitude lower than reported values from other studies. [9, 52, 53] Possible contaminations were checked by preparing a method blank in every analytical batch. No detectable contamination was observed above the MDLs during the experiments related to the present study.

Internal quality control (QC) materials were prepared by spiking liquid human plasma obtained from American Red Cross (Pomona, CA, USA) with inorganic stock standard solutions at two levels. For the low-level QC material, the human plasma was spiked with Cd, W, Hg, Pb and U standards at 0.2 μg L^{-1}. This level was not spiked with Mn and As due to their natural levels in the original pool. For the high-level QC material, another portion of plasma was spiked with all seven analytes at 2 μg L^{-1}. Each pool of the internal QC materials was aliquoted into 3.5 mL cryogenic vials from Perfector Scientific (Atascadero, CA, USA) and stored in a freezer at -20 °C. A set of the two internal QC materials bracketed with CCV/CCB pairs were inserted after the calibration standards and at the end of each analytical batch.

A series of tests for method validation were performed with certified/standard reference materials: animal serum (NIST 1598a) and human serum Seronorm^{TM} Trace Elements Level 1 and 2 from Sero (manufactured in Billingstad, Norway, purchased from Accurate Chemical and Scientific Corporation, Westbury, NY, USA). Finally, proficiency testing specimens were obtained from three rounds of the external quality assessment scheme program of the Institute national de santé publique du Québec (INSPQ).

Results and discussion

Suppression and enhancement effects on analyte signals

The solutions prepared for the experiments to determine enhancing/suppressing effects of organic/inorganic compounds were directly aspired by the nebulizer pump into the ICP-MS instrument. With a He flow rate of 4.5 mL min^{-1} through the collision cell, averages of 1 s integration readings over a period of 1 min were recorded from the tune screen. The sequential readings of the series of solutions were performed twice starting from the lowest concentration to the highest and then in the opposite direction generating pairs of results. Averages of the duplicate measurements were calculated and used for selected graph creation (Figs. 2 through 4).

Figure 2 Effect of n-butanol concentration on signal intensities of carbon, sodium and chloride species. The n-butanol solutions contain n-butanol (various concentrations), 2% w/v NH_4OH, 0.1% w/v of H_4EDTA and 0.1% w/v of Triton X-100.

The relationships between signal intensities of C^+, Na^+ and Cl^+ and the concentration of n-butanol in a mixed solution of n-butanol and NaCl is shown in Fig 2. Considering n-butanol solutions only, e.g., without the presence of NaCl, the C^+ signal intensity was steadily increasing as n-butanol concentration increased up to 8% w/v. Should the cooling effect occur, the C^+ intensity would not increase but decrease rapidly as it was observed for various ionized elements in presence of volatile organic solvents. [41, 42] When NaCl was present in the solution, even the Na^+ signal intensity was noticeably decreased with increasing C^+ concentration due to a much higher total concentration of C and C^+ than Na and Na^+ in the instrument plasma. Simultaneously, C^+ signal intensity was leveling, indicating strong ionic interactions. However, slight decrease in C^+ signal intensity due to the cooling effect from n-butanol on the instrument plasma central channel cannot be entirely excluded.

Figure 3 Effect of n-butanol and Triton X-100 on RSI of analytes and internal standards. The n-butanol solutions contain n-butanol (various concentrations), 2% w/v NH4OH, 0.1% w/v of H_4EDTA and 0.005% (Fig. 3A) or 0.1% (Fig. 3B) w/v Triton X-100. Individual metal concentrations in both solutions are identical: 0.5 µg L^{-1} of Mn, As, Cd, and Pb; 0.05 µg L^{-1} of W, Hg, U and Ga, Rh, Re is Ir 10, 2, 5 and 1 µg L^{-1} respectively. All signal intensities were measured in pulse mode

and normalized to values in solution (RSI) which consists of 2% w/v NH_4OH and 0.1% w/v of H_4EDTA.

The enhancing effect of C on signal intensities, especially for the metalloids such as As or Se, was thoroughly studied over the last few decades. Methanol[23, 28] and mixtures of methanol or acetone[41] as a source of C were found to greatly increase the signal intensities for As and Se. The addition of methanol, ethanol, propanol or butanol had a comparable increasing effect on Se signal intensities[54] as observed in the present study (Fig. 3). The highest sensitivity to the charge transfer effect from C^+ was clearly visible for As with a maximum RSI of ~ 450% when the n-butanol concentration was 2% w/v. This maximum RSI of As is markedly greater than the maximum RSI of 120 – 130% for the other metals, confirming a very low degree of ionization of As without n-butanol. At a higher (>2%) n-butanol concentration, however, noticeable decreases in signal intensities were observed for all analytes, including As and ISTDs. Nevertheless, an n-butanol concentration of 4% w/v was selected to prepare the sample diluent throughout this study, i) to improve the solubility of the plasma samples and ii) to reduce the difference in C concentrations between calibration standards and plasma sample solutions.

Another component of the diluent, Triton X-100, would potentially influence the signal intensities in two ways: i) as a source of C and ii) as a surfactant it would affect nebulization effectiveness. By comparing Fig 3A and 3B, a 20 fold increase in the Triton X-100 concentration from 0.005 to 0.1% had very little effect on the signal intensities of all elements present in the solutions. However, a higher content, 0.1%, of Triton X-100 was used throughout this study to reduce the carryover of any dissolved plasma components, especially functional proteins and nutrients.

The As and/or Se signal enhancement can be also achieved if the C source originated from low molecular weight organic solutes.[28, 54] In the present study, 0.1% w/v of H_4EDTA was used, which is a relatively small amount compared to the n-butanol concentration. It is not expected that this amount would substantially influence the ionization of analytes and ISTDs.

Figure 4 Effect of NaCl concentration on RSI of analytes and ISTDs used in the study. The solution is composed of 4% w/v n-butanol, 2% w/v NH_4OH, 0.1% w/v Triton X-100 and 0.1% w/v H_4EDTA. Metal concentrations in the solutions are: Mn, As, Cd and Pb at 0.5 µg L^{-1}; W, Hg and U at 0.05 µg L^{-1}; Ga, Rh, Re and Ir is 10, 2, 5 and 1 µg L^{-1} respectively. All signal intensities were measured by detector in pulse mode and normalized to values in solution which consisted of 2% w/v NH_4OH and 0.1% w/v H_4EDTA.

The effect of Na^+ concentration on signal intensities in a calibration standard is shown in Fig. 4. The signal intensity values were normalized to the values acquired from solution with 2% w/v NH_4OH and 0.1% w/v H_4EDTA. After rapid non-linear decreases in RSI with increasing NaCl concentration from 0 to 0.1%, the descent rate became near linear at higher NaCl concentrations. Noticeably, the suppression effects of NaCl for concentrations > 0.1% were

comparable for most elements, except for ^{55}Mn and ^{71}Ga (dashed lines) where the effect was less. The differences were probably caused by known polyatomic interferences for ^{55}Mn and ^{71}Ga which would increase their respective intensities, e.g., $^{37}Cl^{18}O^+$ and $^{37}Cl^{17}OH^+$ for m/z 55 and $^{35}Cl^{18}O_2^+$, $^{37}Cl^{16}O^{18}O^+$, $^{37}Cl^{17}O_2^+$ and $^{36}Ar^{35}Cl^+$ for m/z 71. For ^{75}As, there are known interferences, e.g., $^{40}Ar^{35}Cl^+$ and $^{38}Ar^{37}Cl^+$, but the suppression effect of NaCl overwhelms the possible signal enhancement, causing a jointly opposite decreasing effect. (Note that the y-axis scale is different for As). In general, the polyatomic interferences are not entirely removed by He gas in the collision cell but remaining fractions appeared to cause only small increase in their respective backgrounds. For instance, the m/z 75 signal in the 0.1 µg L^{-1} calibration standard solution resulted in a signal intensity of ~ 1000 cps whereas signal intensity in the blank was 10 cps. For the rest of the elements, considering their respective m/z values, there are no other known polyatomic interferences containing Cl, suggesting that the correlations reflect only the suppressing effect of the NaCl.

The equivalency in the role of n-butanol (when > 2%) (Fig. 3A and 3B) and NaCl (Fig. 4), (n-butanol and NaCl after ionization are mainly C^+, Na^+ and Cl^+, Fig 2), in affecting the elemental signal intensities suggests that a thermodynamic equilibrium-based model[39] would more accurately describe the relationships between components in the calibration standard than the cooling effect. Under plasma condition, an equilibrium concentration of all plasma components (atoms, ions and electrons) can be established at any given moment. The individual element ion-atom equilibrium can be changed with concentrations of other ionized or atomic plasma constituents. At equilibrium, degree of ionization of an element is determined by components at their highest concentrations, in our case C^+ and Na^+. Although concentrations of N (mainly from NH_4OH) and Cl (mainly from NaCl) are relatively high, concentrations of N^+ and Cl^+ will be relatively low due to their very high FIPs, 14.53 and 12.97 eV, respectively. Consequently, as in our study, at a constant n-butanol concentration in calibration standards, the degree of ionization of all elements present can be achieved by adjusting the NaCl concentration. Conversely, at a constant NaCl concentration, degree of ionization of the elements could be changed by altering the C concentration coming not only from n-butanol but from any other organic compounds.

Effects of matrix-match calibration on analytical results

As indicated earlier, unequal suppression of the analytes in calibration standards and plasma samples may considerably bias the accuracy of analytical results. In addition, the selection of ISTDs to compensate for these inequalities can be quite challenging as indicated in previous studies.[49, 50]

Human plasma contains about 40 g L^{-1} of C.[36] Most of the C is contained in proteins, e.g., albumins, globulins and fibrinogen. If the molecular weight of the solute is too high, the solute fragments can survive the instrument plasma and the incomplete atomization can be explained by the chemical structure and the insufficient residence time of the solute in the instrument plasma.[55] The fragmented components of the human plasma even with the m/z identical to the analytes or ISTD most likely would be removed by the collision cell as polyatomic interferences and not affect suppression or enhancement of their signal intensities. The C from atomized bio-organic plasma components is likely to cause a similar effect as the C in n-butanol. For the present study, a dilution factor of 10 was applied, resulting in an approximate protein C concentration of 4 g L^{-1} in the solution. For comparison, 4% of n-butanol in the diluent contained about 24 g L^{-1} C. Aside from C, human plasma consists of multiple elements contained in its inorganic and organic components. Elements such as S, P and N (N is also existing as NH_4^+ in the diluent) would have little or no effect on the analytes and ISTDs ionization due to their high FIPs of 10.36, 10.49 and 14.53 eV, respectively.

It has been suggested that individual elemental ionization suppression depends on the total concentration of concomitant salt and ionization energies of its elemental components.[39] This finding is correct in a general sense, but in the present study, 2% n-butanol produced an optimal amount of C^+ (Fig. 3) where all the elements, including those with high FIPs, were likely to be fully ionized. Additional amounts of n-butanol and/or NaCl caused suppression of the ionization (Fig. 2 and 3) and all elements behaved very similarly regardless of their FIP or atomic mass. Hereafter, it can be hypothesized that the collective effect of all EIE present in human plasma, such as K, Na, Ca and Mg with FIP 4.34, 5.14, 6.11 and 7.64 eV respectively, should have a similar effect on ionization suppression equivalent to the effect of Na concentration alone. Finally, added Na should compensate for the suppression effect of organic and inorganic C introduced with the human plasma. Recently, we have demonstrated the usefulness of the “synthetic matrix” addition to calibration standards, where the mixture of NaCl and $CaCl_2$ fully compensated for the difference between the elemental ionization in calibration standards and blood sample solutions.[51]

Direct injection of diluted plasma samples is an attractive alternative to requiring heat/chemical or enzymatic digestion prior to injection; it is time-efficient and is less susceptible to contamination. However, an undigested plasma solution contains bio-organic solutes, organic solvents (if used for the diluent preparation) and inorganic serum components. Under these conditions, both signal enhancement and suppression effects occur when the solution is injected into the ICP-MS. Because both effects influence the individual elements at different degrees, it is difficult to predict the behavior of

the final analyte signal intensities. Moreover, to achieve reasonable detectability for most ultra-trace elements, the final dilution of serum samples should not exceed 10 folds. Consequently, concentrations of all matrix components will be at relatively high levels, and there are no simple answers for eliminating or quantifying the ME.[43] Despite the ME challenge, we have observed some analyte behaviors during designed experiments which were helpful in interpreting the results and proposing ways to minimize some adverse effects of the ME.

Fig. 5 illustrates how the stability of the ISTDs was influenced by the addition of NaCl to the calibration standards. Several human plasma specimens were prepared in triplicate (labeled R1, R2 and R3) by 1:10 dilution with the sample diluent and then analyzed with three sets of calibration standards. The only variable in the three analytical batches was the concentration of NaCl in the working calibration standards, carrier solution, CCV and CCB, i.e., 0.0 % w/v (Fig. 5A), 0.1% w/v (Fig. 5B) and 0.12% w/v (Fig. 5C). The best matrix match, estimated by the variability of ISTDs in samples, CCVs and CCBs, was achieved when the concentration of NaCl was 0.10% (Fig. 5B). Relatively low signal intensities of the ISTDs were observed during the analysis of the plasma specimens, ranging approximately from 91 to 95 %, compared to those for calibrations standards and CCV/CCB (Fig. 5B). This suppression may be attributed to the additional C (~ 4 g L^{-1}) supplied from bio-organic components in plasma specimens and the related suppression effect by C^+, as discussed in Fig. 3. However, slight decrease in the RSI due to diminished effectiveness of nebulization caused by different physicochemical properties, e.g., higher viscosity, density and surface tension in plasma solution, compared to calibrations standards, cannot be completely excluded.

For the NaCl concentrations of 0.0% and 0.12%, however, considerable instability of ISTD and differences between individual ISTD RSI were noted (Fig. 5A and 5C). Even if the carrier solution rinsed the sample pathway between plasma sample injections, there was not enough time for a complete wash out of the matrix components from the sample introduction parts of the ICP-MS. Consequently, signal suppressing components of the matrix were slowly accumulating after each injection and the ISTD signal intensities gradually decreased. Noticeably, all the CCVs and CCBs, as observed in Fig. 5A, had lower signal intensities than the calibration blank (all did not contain NaCl), confirming increased suppression of all ISTDs signal intensities caused by carryover of matrix suppression components present in the plasma specimens. Conversely, a slim overall upward trend was observed in Fig. 5C with a noticeable increase in RSI for the last two sets of CCB/CCV. Here at the beginning of the run, the instrument sample pathway was equilibrated with 0.12% NaCl. After subsequent plasma sample injections, the NaCl concentration of the solution retained in the ICP-MS sample pathway

becomes gradually diluted. As a consequence, the RSI increased accordingly. For comparison, a transient effect is observed when 0.1% NaCl is injected to the ICP-MS, and even after rinsing the instrument with DI water for 10 min, the Na signal intensity would only decrease by 50%.

Figure 5 Comparison of ISTDs RSI during analytical runs with different NaCl concentrations in calibration standards. The RSI are ratios of elemental signal intensities in a sample and the calibration blank. The human plasma specimens # 1 and 2, QC Low and QC High samples were analyzed in triplicates labeled R1, R2 and R3. The only variable in all three analytical runs (A, B & C) was the concentration of NaCl in calibration standards. All plasma specimens and calibration standards were diluted 10 times with the diluent. Therefore an actual concentration of NaCl in working calibration standard solutions reaching the instrument plasma was 10 times lower (i.e., Fig 5B and 5C were 0.1 and 0.12% w/v, respectively). Boxes on Fig 5A and B indicate data discussed in Fig 6A and 6B, respectively.

The matrix-match condition, when the working calibration standards and carrier solution contain 0.1% NaCl, efficiently eliminated the transient effects (Fig. 5B), indicating an equivalency of NaCl added to the calibration standards and matrix components present in human serum. Introduction of any EIE into the instrument plasma would suppress ionization not only due to plasma ion-atom equilibrium in the central plasma channel but also due to the space charge effect taking place in different part of the ICP-MS ionic pathway, e.g., behind skimmer cone in ion lenses. [25, 31, 56] A shift between RSI for lower mass ISTDs, ^{71}Ga and ^{103}Rh, from the high mass ISTDs,^{185}Re and ^{193}Ir, occurred for all the samples and became quite apparent for the CCB and CCV (Fig. 5A). Such shift would be consistent with the space charge effect caused by presence of the EIE.[38, 56] Although all non-plasma samples do not contain NaCl, carryover and accumulation of EIE sourcing from the plasma samples occurred, as discussed earlier. Again, if there is matrix-match between calibration standards and sample matrix, the space charge effect would be compensated and the change in the RSI would be minimized as depicted in Fig 5B.

Figure 6 Comparison of mean concentrations of seven analytes without (A) and with (B) 1% of NaCl in calibration standards. Each bar represents an average of three replicate results (R1, R2 and R3) of QC High samples (as shown in Figure 5A and 5B). The calculations were performed for each of the four ISTDs used in the study. The coefficient of variance (CV), % values calculated for each analyte are shown above the bars.

The triplicate runs for a plasma specimen, i.e., QC High R1 through R3, boxed in Fig. 5A and 5B were quantitatively re-calculated using the four ISTDs with various masses and FIP (^{71}Ga, 6.00 eV; ^{103}Rh, 7.46 eV; ^{185}Re, 7.87 eV; ^{193} Ir, 9.2 eV), averaged and compared in Fig. 6A and 6B, respectively. When NaCl was not added to the calibration standards (Fig 5A), the concentration of an analyte considerably varied depending on the ISTD used for calculation (Fig. 6A). The As concentration, for instance, was 3.25 µg L^{-1} when quantified with ^{185}Re, increased to 3.54 µg L^{-1} when re-calculated with ^{103}Rh. The coefficient of variability (CV) of the four re-calculated values averaged 3.5% for all the analytes, which can lead to a possible bias in analytical results when there is difficulty to choose an appropriate ISTD for an

analyte. Fig. 6B clearly shows that this "ISTD-dependent" variability became negligible when 1% of NaCl was added to the intermediate standards. The CV of the four re-processed values averaged 0.57% and the ISTD-dependent bias that persistently observed in Fig. 6A was minimal. These results suggest that with the current matrix-match approach, any ISTD can be selected for any analyte regardless of FIP or atomic mass (i.e., ^{71}Ga is an acceptable ISTD for ^{238}U or ^{193}Ir is a good ISTD for ^{55}Mn, etc.) and the use of a single ISTD might be sufficient enough to compensate for the difference in physicochemical properties between calibration standards and plasma/serum samples.

Method validation

Table 4 Analytical results of various reference materials

Table 4 presents the analytical results from repeat analyses of certified/standard reference materials: two levels of Seronorm™ human serum, NIST 1598A animal serum, and INSPQ proficiency testing (PT) serum samples. The recoveries from all of the reference materials were within 100 ± 25 % with a few exceptions: As in Seronorn level 1 (155 %), U in Seronorm level 2 (150 %) and Cd in NIST 1598A (141 %). The most striking discrepancy was observed from Hg in NIST 1598A, with an average recovery of 8.7% from two vials. The reason for the low recovery of Hg is unclear. However, the matrix spike recoveries of Hg, tested with NIST 1598A at 0.2 and 2.0 μg L^{-1}, averaged 91.3 ± 4.0 % (n = 6), suggesting that the low recovery of 8.7% likely did not result from an analytical bias. It is interesting to note that, from both Seronorm level 1 and 2, As and W had substantially greater inter-vial (between vials) variability than the other analytes, with CV values ranging from 13.7 to 30.6%. Intra-vial (within a vial) variability for As and W were, however, minimal with CV values ranging from 0.7 to 4.9% (intra-vial CV calculations not shown in Table 5). This variability can probably be explained by suspected incomplete homogenization of the elemental components during manufacturing processes. A similar inter-vial difference was reported by a previous study[53] for the same metals measured in the human serum Seronorm. The best agreement between certified and analytical results was found in the INSPQ PT samples. All results were within ±10% of the certified/reference values with the exception of the Hg result for sample Q1304 which had a recovery of 87%. The low recoveries of Hg probably resulted from the overlap of $^{186}W^{16}O^{+}$ on the $^{202}Hg^{+}$ signal in calibration standards, which may become significant when the concentration ratio of W to Hg in a plasma specimen is remarkably lower (e.g., < 0.1) than the ratio in calibration standards (1:1). Our preliminary tests demonstrated that increasing the He flow rate up to 6.5 mL min^{-1} did not eliminate $^{186}W^{16}O^{+}$ but only decreased ^{202}Hg signal by 70%. An alternative solution to this bias may be to monitor ^{201}Hg, which is less abundant (13.2%), instead of ^{202}Hg (29.9%) as it

is free from the W interference. In order to use ^{201}Hg, however, Re should not be used as an internal standard to avoid the $^{185}Re^{16}O^{+}$ interference on the $^{201}Hg^{+}$ signal.

Table 5 Statistical evaluation of the internal quality control human plasma reference materials

Two levels of the internal QC materials were prepared and analyzed as a part of our routine procedures. A statistical evaluation of analytical results from repeat analyses of each QC level is provided in Table 5. The long term stabilities over a period of 50 days, in terms of CV, for all the metals were between 1.5 and 3.2% for both levels. The daily reproducibility or relative percent difference was < 7% for the low-level QC samples and < 3% for the high-level QC samples. Storage stability of the QC materials was evaluated at two temperatures (+4 and -20^{o}C) over a period of 50 days and no meaningful concentration change was observed for any analyte.

Conclusions

Our method for determination of metals and metalloids in human plasma/serum by direct injection of a diluted sample into an ICP-MS with ISIS-DS is relatively rapid, accurate and precise. The optimal diluent is a solution of 4% w/v n-butanol, 2% w/v NH_4OH, 0.1% w/v Triton X-100 and 0.1% w/v H_4EDTA. The 4% w/v of n-butanol not only keeps bio-organic components of plasma/serum in solution, but it also plays an important function as a significant source of C, which is necessary to achieve an enhancing effect on ionized metals in the instrument plasma. Additional amount of C above 2% however, causes signal suppression rather than enhancing elemental signals. The addition of 1% NaCl to the intermediate calibration standards, i.e., 0.1% in the working calibration standard solutions, successfully provides a matrix-match effect and compensates for effect of inorganic components present in human plasma. Na added to the calibration standards in presence of C compensates not only for the combined suppressing effect by EIE on analytes and ISTD present in the plasma solutions but also for the space charge effect in the instrument ion lenses.

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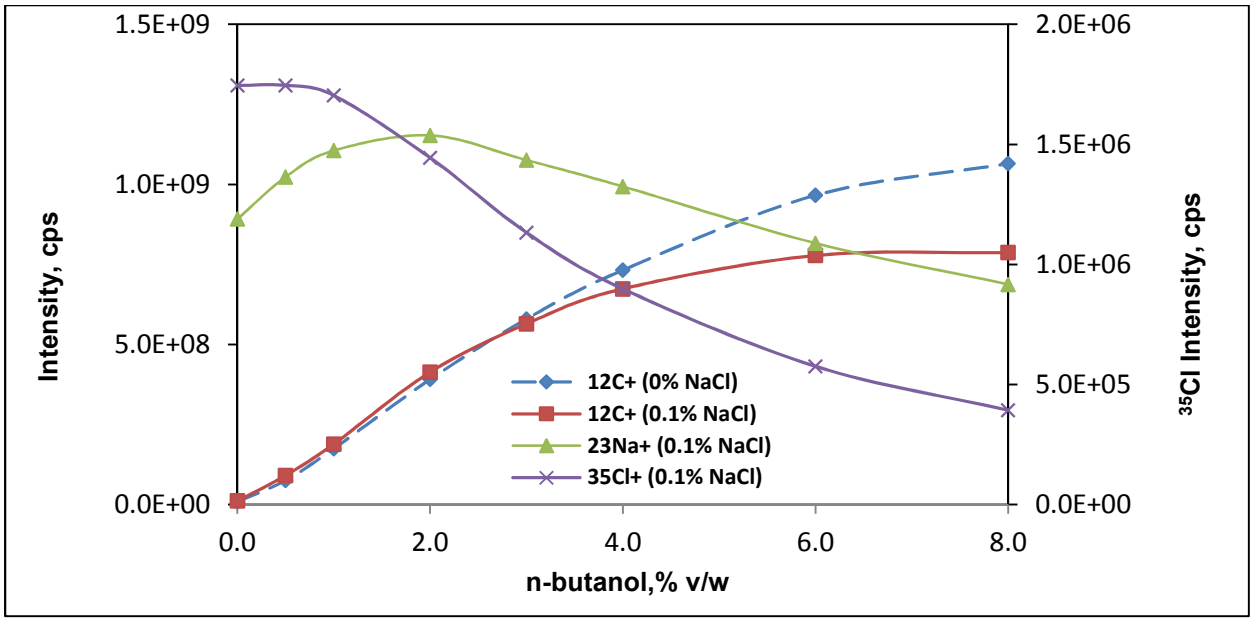
References

1. N. Psychogios, D. D. Hau, J. Peng, A. C. Guo, R. Mandal, S. Bouatra, I. Sinelnikov, R. Krishnamurthy, R. Eisner, B. Gautam, N. Young, J. Xia, C. Knox, E. Dong, P. Huang, Z. Hollander, T.

L. Pedersen, S. R. Smith, F. Bamforth, R. Greiner, B. McManus, J. W. Newman, T. Goodfriend and D. S. Wishart, *PloS one*, 2011, **6**, e16957. DOI: 10.1371/journal.pone.0016957.
2. B. Bocca, D. Mattei, A. Pino and A. Alimonti, *Rapid communications in mass spectrometry : RCM*, 2010, **24**, 2363-2369. DOI: 10.1002/rcm.4650.
3. B. Bocca, D. Mattei, A. Pino and A. Alimonti, *Rapid communications in mass spectrometry : RCM*, 2011, **25**, 453-458. DOI: 10.1002/rcm.4852.
4. P. Hoet, E. Vanmarcke, T. Geens, G. Deumer, V. Haufroid and H. A. Roels, *Toxicology letters*, 2012, **213**, 69-74. DOI: 10.1016/j.toxlet.2011.06.013.
5. I. Rodushkin, E. Engström, A. Stenberg and D. C. Baxter, *Analytical & Bioanalytical Chemistry*, 2004, **380**, 247-257. DOI: 10.1007/s00216-004-2742-7.
6. L. Balcaen, E. Bolea-Fernandez, M. Resano and F. Vanhaecke, *Anal Chim Acta*, 2014, **809**, 1-8. DOI: 10.1016/j.aca.2013.10.017.
7. R. Forrer, K. Gautschi and H. Lutz, *Biol Trace Elem Res*, 2001, **80**, 77-93. DOI: 10.1385/bter:80:1:77.
8. J. P. Goulle, L. Mahieu, J. Castermant, N. Neveu, L. Bonneau, G. Laine, D. Bouige and C. Lacroix, *Forensic Sci Int*, 2005, **153**, 39-44. DOI: S0379-0738(05)00208-2 [pii]

10.1016/j.forsciint.2005.04.020.
9. A. Cesbron, E. Saussereau, L. Mahieu, I. Couland, M. Guerbet and J. P. Goulle, *J Anal Toxicol*, 2013, **37**, 401-405. DOI: 10.1093/jat/bkt046.
10. E. Barany, I. A. Bergdahl, A. Schutz, S. Skerfving and A. Oskarsson, *Journal of Analytical Atomic Spectrometry*, 1997, **12**, 1005-1009. DOI: 10.1039/a700904f.
11. R. Wahlen, L. Evans, J. Turner and R. Hearn, *Agilent Application - Clinical*, 2005.
12. D. Smith, M. Hernandez-Avila, M. M. Téllez-Rojo, A. Mercado and H. Hu, *Environmental health perspectives*, 2002, **110**, 263-268.
13. T. Zhao, T. Chen, Y. Qiu, X. Zou, X. Li, M. Su, C. Yan, A. Zhao and W. Jia, *Anal Chem*, 2009, **81**, 3683-3692. DOI: 10.1021/ac900311q.
14. M. Su, T. Zhang, T. Zhao, F. Li, Y. Ni, X. Wang, T. Chen, A. Zhao, Y. Qiu, Y. Bao, W. Jia and W. Jia, *Metallomics : integrated biometal science*, 2012, **4**, 244-252. DOI: 10.1039/c2mt00178k.
15. P. Kodali, K. R. Chitta, J. A. Landero Figueroa, J. A. Caruso and O. Adeoye, *Metallomics : integrated biometal science*, 2012, **4**, 1077-1087. DOI: 10.1039/c2mt20092a.
16. I. Knerr, H. Blessing, S. Seyferth, R. J. Watling and M. A. Chaudhri, *Annals of nutrition & metabolism*, 2013, **63**, 168-173. DOI: 10.1159/000354869.
17. G. Li, *American Journal of Analytical Chemistry*, 2012, **03**, 646-650. DOI: 10.4236/ajac.2012.39084.
18. Q. Pasha, S. A. Malik, J. Iqbal, N. Shaheen and M. H. Shah, *Human and Ecological Risk Assessment: An International Journal*, 2009, **15**, 1016-1032. DOI: 10.1080/10807030903153188.
19. F. R. Abou-Shakra, M. P. Rayman, N. I. Ward, V. Hotton and G. Bastian, *Journal of Analytical Atomic Spectrometry*, 1997, **12**, 429-433. DOI: 10.1039/a607972e.
20. B. L. Batista, J. L. Rodrigues, J. A. Nunes, L. Tormen, A. J. Curtius and F. Barbosa Jr, *Talanta*, 2008, **76**, 575-579. DOI: http://dx.doi.org/10.1016/j.talanta.2008.03.046.
21. E. R. Verni, F. Moyano, L. D. Martinez, A. V. Lapierre and R. A. Gil, *Journal of Analytical Atomic Spectrometry*, 2013, **28**, 1655-1659. DOI: 10.1039/C3JA50191D.
22. P. Allain, L. Jaunault, Y. Mauras, J. M. Mermet and T. Delaporte, *Analytical Chemistry*, 1991, **63**, 1497-1498. DOI: 10.1021/ac00014a028.
23. E. H. Larsen and S. Sturup, *Journal of Analytical Atomic Spectrometry*, 1994, **9**, 1099. DOI: 10.1039/ja9940901099.
24. G. H. Floor, R. Millot, M. Iglesias and P. Négrel, *Journal of Mass Spectrometry*, 2011, 182-188. DOI: 10.1002/jms.1880.
25. C. Mariet, F. Carrot and M. Moskura, *American Journal of Analytical Chemistry*, 2011, **02**, 739-751. DOI: 10.4236/ajac.2011.27085.
26. C. T. Gross, S. M. McIntyre and R. S. Houk, *Analytical Chemistry*, 2009, **81**, 4898-4905. DOI: 10.1021/ac900568x.
27. R. Xie, W. Johnson, S. Spayd, G. S. Hall and B. Buckley, *Analytica Chimica Acta*, 2006, **578**, 186-194. DOI: 10.1016/j.aca.2006.06.076.
28. P. Kralj and M. Veber, *Acta chimica slovenica*, 2003, 633-644.
29. K.-S. Park, S.-T. Kim, Y.-M. Kim, Y. Kim and W. Lee, *Bulletin of the Korean Chemical Society*, 2002, **23**, 1389-1393. DOI: 10.5012/bkcs.2002.23.10.1389.
30. H. Ying, M. Antler, J. W. Tromp and E. D. Salin, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2002, **57**, 277-290. DOI: http://dx.doi.org/10.1016/S0584-8547(01)00382-2.

31. M. M. Fraser and D. Beauchemin, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2000, **55**, 1705-1731. DOI: http://dx.doi.org/10.1016/S0584-8547(00)00273-1.
32. M. M. Fraser and D. Beauchemin, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2001, **56**, 2479-2495. DOI: http://dx.doi.org/10.1016/S0584-8547(01)00346-9.
33. I. I. Stewart and W. J. Olesik, *Journal of Analytical Atomic Spectrometry*, 1998, **13**, 1313-1320.
34. A. A. Pupyshev, N. L. Vasil'eva and S. V. Golik, *J Appl Spectrosc*, 1998, **65**, 804-811. DOI: 10.1007/bf02679850.
35. C.-S. Hsiung, J. D. Andrade, R. Costa and K. O. Ash, *Clinical Chemistry*, 1997, **43**, 2303-2311.
36. C. Vandecasteele, H. Vanhoe and R. Dams, *Journal of Analytical Atomic Spectrometry*, 1993, **8**, 781-786.
37. S. H. Tan and G. Horlick, *Journal of Analytical Atomic Spectrometry*, 1987, **2**, 745-763.
38. H. Kawaguchi, T. Tanaka and A. Mizuike, *Analytical Sciences*, 1987, **3**, 305-308.
39. J. A. Olivares and R. S. Houk, *Analytical Chemistry*, 1986, **58**, 20-25. DOI: 10.1021/ac00292a008.
40. C. Bonnefoy, A. Menudier, C. Moesch, G. Lachâtre and J. M. Mermet, *Anal Bioanal Chem*, 2005, **383**, 167-173. DOI: 10.1007/s00216-005-3403-1.
41. Z. Hu, S. Hu, S. Gao, Y. Liu and S. Lin, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2004, **59**, 1463-1470. DOI: http://dx.doi.org/10.1016/j.sab.2004.07.007.
42. Z. C. Hu, S. Gao, S. H. Hu, Y. S. Liu and H. H. Chen, *Chinese Chemical Letters*, 2007, **18**, 1297-1300. DOI: http://dx.doi.org/10.1016/j.cclet.2007.08.012.
43. C. Agatemor and D. Beauchemin, *Analytica Chimica Acta*, 2011, **706**, 66-83. DOI: http://dx.doi.org/10.1016/j.aca.2011.08.027.
44. A. S. Al-Ammar, E. Reitznerová and R. M. Barnes, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 1999, **54**, 1813-1820. DOI: http://dx.doi.org/10.1016/S0584-8547(99)00124-X.
45. C. Richardson, E. Roberts, S. Nelms and N. B. Roberts, *Clinical chemistry and laboratory medicine : CCLM / FESCC*, 2012, **50**, 317-323. DOI: 10.1515/CCLM.2011.775.
46. B. Bocca, D. Mattei, A. Pino and A. Alimonti, *Ann Ist Super Sanita*, 2010, **46**, 259-265. DOI: DOI: 10.4415/ANN_10_03_06.
47. J. F. Rosen and E. E. Trinidad, *Environmental health perspectives*, 1974, **7**, 139-144.
48. C. Muñiz, J. Fernández-Martin, J. Marchante-Gayón, J. Alonso, J. Cannata-Andía and A. Sanz-Medel, *Biol Trace Elem Res*, 2001, **82**, 259-272. DOI: 10.1385/bter:82:1-3:259.
49. H. J. Finley-Jones and J. A. Holcombe, *Journal of Analytical Atomic Spectrometry*, 2009, **24**.
50. H. J. Finley-Jones, J. L. Molloy and J. A. Holcombe, *Journal of Analytical Atomic Spectrometry*, 2008, **23**, 1214-1222.
51. R. Gajek, F. Barley and J. She, *Analytical Methods*, 2013, **5**, 2193-2202. DOI: 10.1039/c3ay26036d.
52. E. Bárány, I. A. Bergdahl, L.-E. Bratteby, T. Lundh, G. Samuelson, A. Schütz, S. Skerfving and A. Oskarsson, *Science of The Total Environment*, 2002, **286**, 129-141. DOI: http://dx.doi.org/10.1016/S0048-9697(01)00970-6.
53. J.-P. Goullé, L. Mahieu, J. Castermant, N. Neveu, L. Bonneau, G. Lainé, D. Bouige and C. Lacroix, *Forensic Science International*, 2005, **153**, 39-44. DOI: http://dx.doi.org/10.1016/j.forsciint.2005.04.020.
54. I. Llorente, M. Gómez and C. Cámara, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 1997, **52**, 1825-1838. DOI: 10.1016/s0584-8547(97)00067-0.
55. V. F. Taylor, R. E. March, H. P. Longerich and C. J. Stadey, *International Journal of Mass Spectrometry*, 2005, **243**, 71-84. DOI: http://dx.doi.org/10.1016/j.ijms.2005.01.001.
56. S. D. Tanner, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 1992, **47**, 809-823. DOI: http://dx.doi.org/10.1016/0584-8547(92)80076-S.



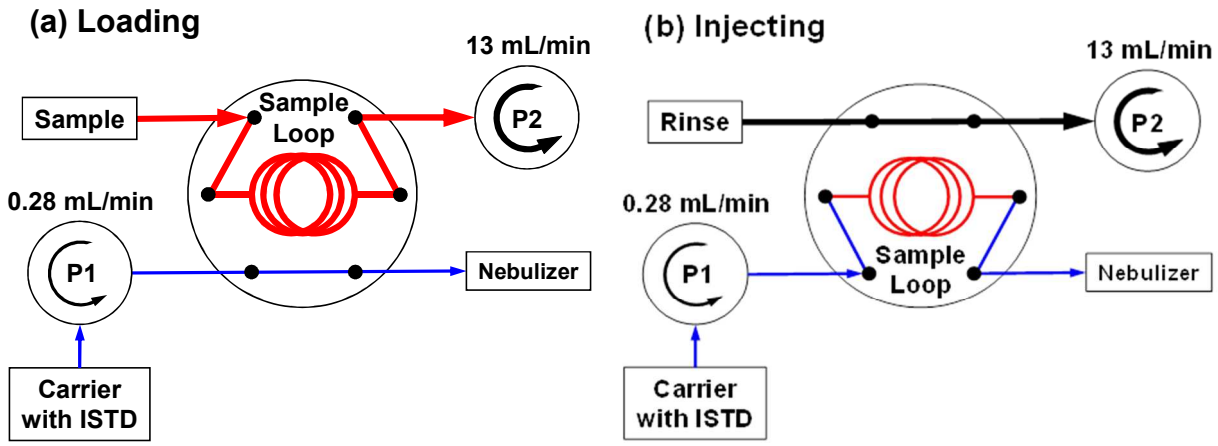


Figure 1 Schematic diagram of solution flow in the ISIS during a) loading and b) injecting mode. In all experiments, the carrier solution composition was identical to the diluted blank used in the specific run.

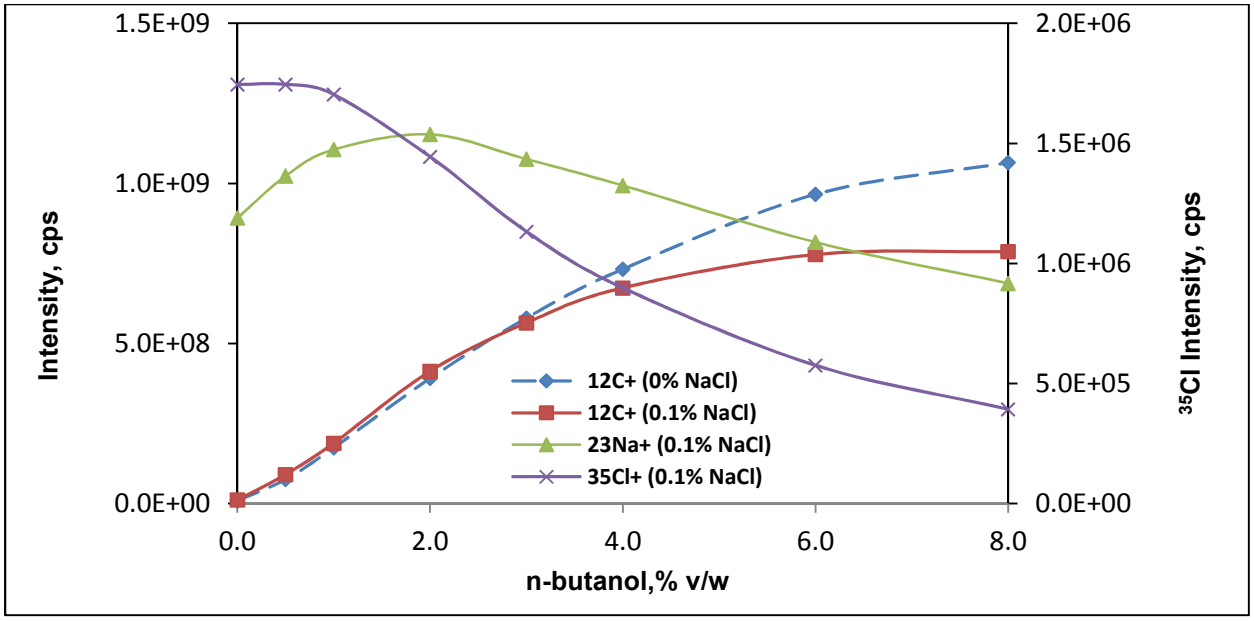


Figure 2 Effect of n-butanol concentration on signal intensities of carbon, sodium and chloride ions. The n-butanol solutions contain n-butanol (various concentrations), 2% w/v NH4OH, 0.1% w/v of H4EDTA and 0.1% w/v of Triton X-100.

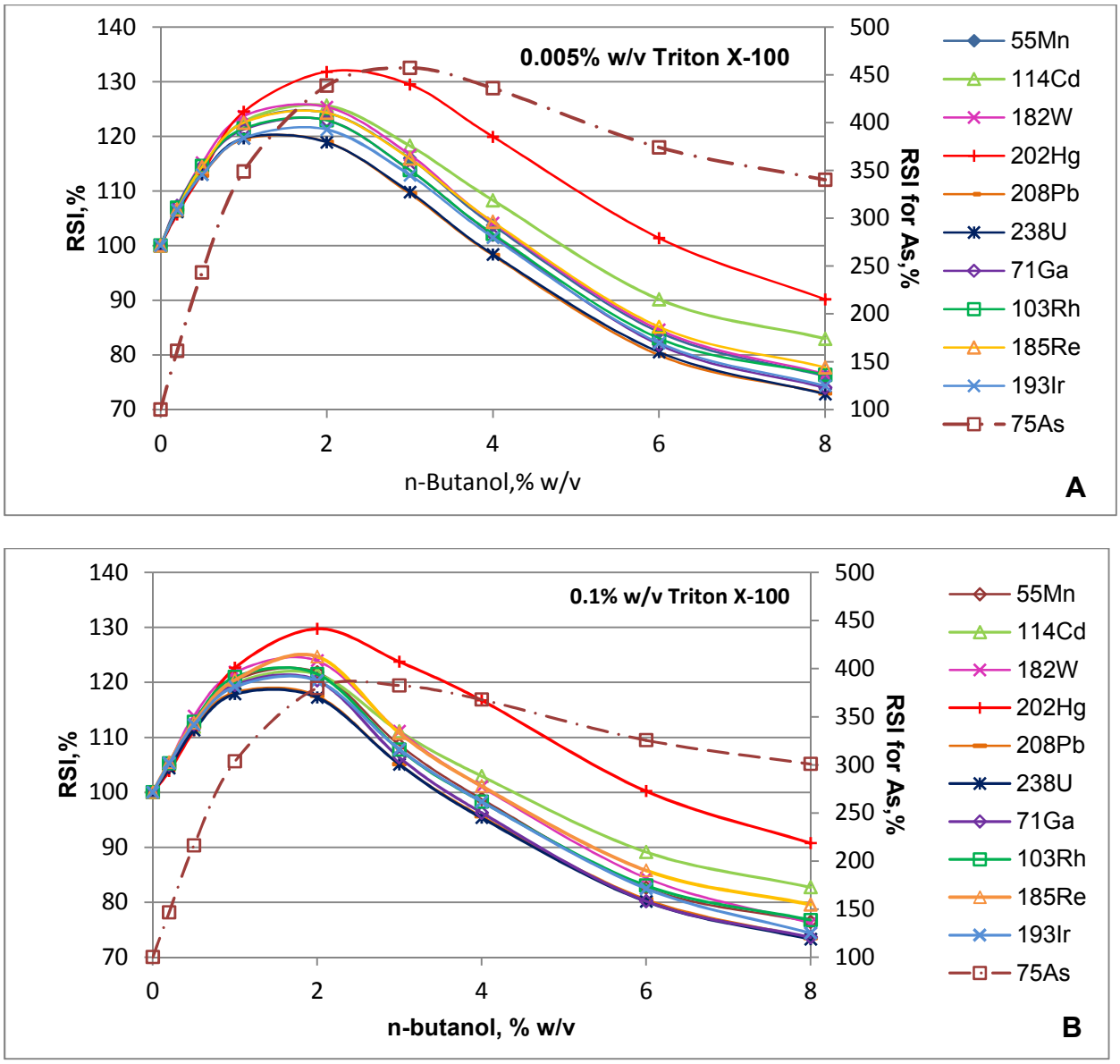


Figure 3 Effect of n-butanol and Triton X-100 on RSI of analytes and internal standards. The n-butanol solutions contain n-butanol (various concentrations), 2% w/v NH4OH, 0.1% w/v of H4EDTA and 0.005% (Fig. 3A) or 0.1% (Fig. 3B) w/v Triton X-100. Individual metal concentrations in both solutions are identical: 0.5 µg L-1 of Mn, As, Cd, and Pb; 0.05 µg L-1 of W, Hg, U and Ga, Rh, Re is Ir 10, 2, 5 and 1 µg L-1 respectively. All signal intensities were measured in pulse mode and normalized to values in solution (RSI) which consists of 2% w/v NH4OH and 0.1% w/v of H4EDTA.

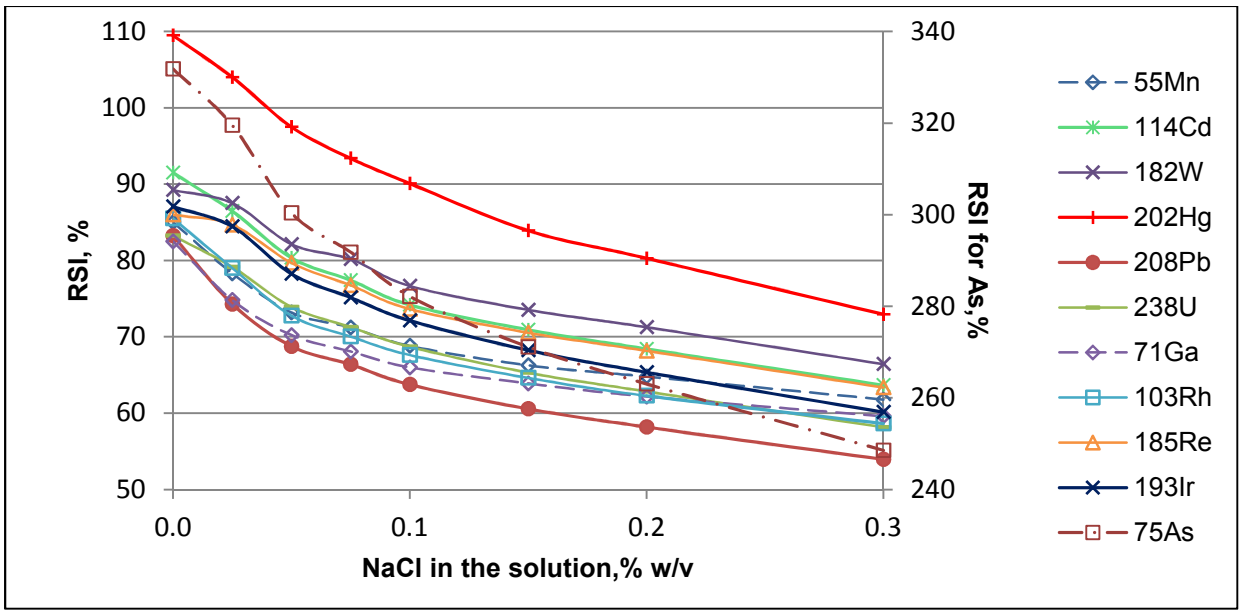


Figure 4 Effect of NaCl concentration on RSI of analytes and ISTDs used in the study. The solution is composed of 4% w/v n-butanol, 2% w/v NH4OH, 0.1% w/v Triton X-100 and 0.1% w/v H4EDTA. Metal concentrations in the solutions are: Mn, As, Cd and Pb at 0.5 µg L-1; W, Hg and U at 0.05 µg L-1; Ga, Rh, Re and Ir is 10, 2, 5 and 1 µg L-1 respectively. All signal intensities were measured by detector in pulse mode and normalized to values in solution which consisted of 2% w/v NH4OH and 0.1% w/v H4EDTA.

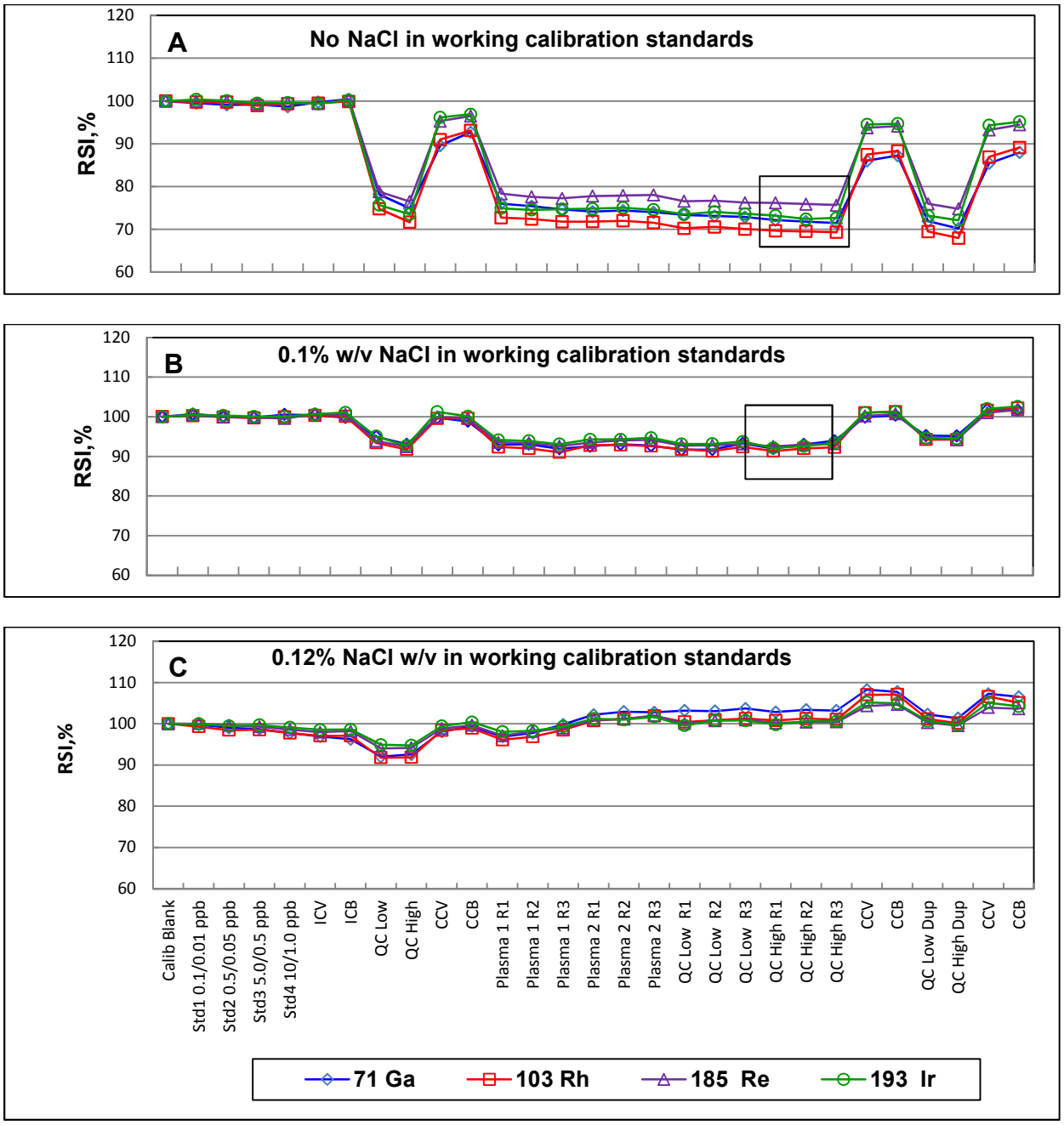


Figure 5 Comparison of ISTDs RSI during analytical runs with different NaCl concentrations in calibration standards. The RSI are ratios of elemental signal intensities in a sample and the calibration blank. The human plasma specimens # 1 and 2, QC Low and QC High samples were analyzed in triplicates labeled R1, R2 and R3. The only variable in all three analytical runs (A, B & C) was the concentration of NaCl in calibration standards. All plasma specimens and calibration standards were diluted 10 times with the diluent. Therefore an actual concentration of NaCl in working calibration standard solutions reaching instrument plasma was 10 times lower (i.e. Fig 5B and 5C were 0.1 and 0.12% w/v, respectively). Boxes on Fig 5A and 5B indicate data discussed in Fig 6A and 6B, respectively.

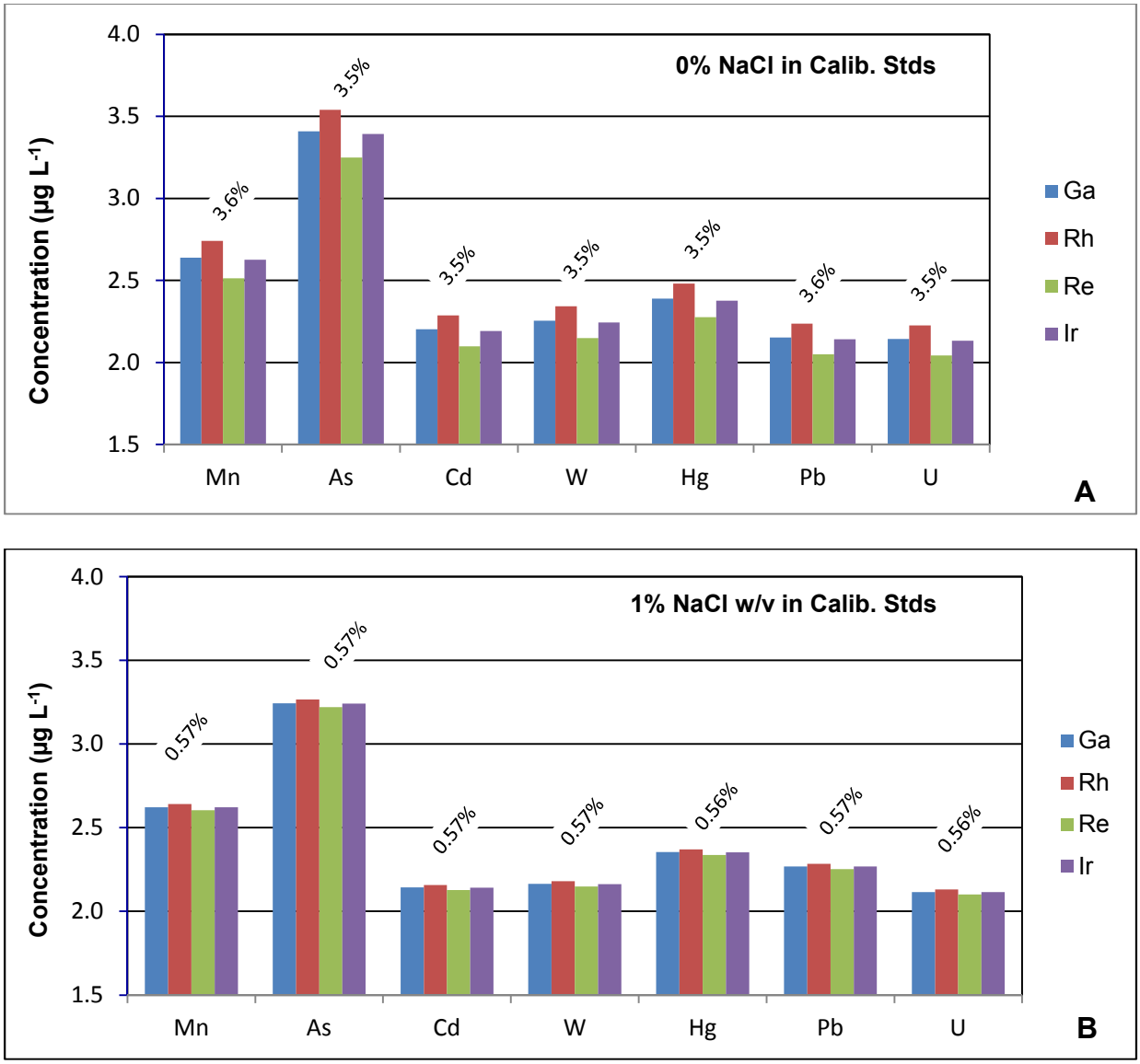


Figure 6 Comparison of mean concentrations of seven analytes without (A) and with (B) 1% of NaCl in calibration standards. Each bar represents an average of three replicate results (R1, R2 and R3) of QC High samples (as shown in Figure 5A and 5B). The calculations were performed for each of the four ISTDs used in the study. The coefficient of variance (CV), % values calculated for each analyte are shown above the bars.

Table 1 Agilent 7700x operating parameters

ICP-MS	Setting	
RF power	1550	W
Number of points per peak	6	
Carrier gas	1.04	L min^{-1}
Dilution gas	0.1	L min^{-1}
He gas	4.5	mL min^{-1}
ISIS-DS	**Setting**	
Load time	10	s
Load speed	1.0	rps
Probe rinse time/Read delay	35	s
Post rinse time	40	s
Post rinse speed	0.8	rps
Sample loop tubing length	120	cm
Loop tubing ID	0.8	mm
Sample loop volume	600	µL

Table 2 Spectrum acquisition parameters

Mass Element	Integration Time per Mass, s
^{55}Mn	1.5
^{71}Ga	1.0
^{75}As	2.1
^{103}Rh	0.70
^{111}Cd	3.2
^{182}W	0.70
^{185}Re	0.70
^{202}Hg	3.2
^{205}Ir	0.70
^{206}Pb	1.1
^{207}Pb	1.1
^{208}Pb	1.1
^{238}U	1.2

Acquisition time: 18.3 s, 3 repetitions
Total acquisition time: 54.9 s
Total Pb = (208)*1 + (206)*1+ (207)*1

Table 3 Metal concentrations in the working calibration standards

Level	As, Mn, Cd, Pb, µg L^{-1}	W, Hg, U, µg L^{-1}
1	0.00	0.00
2	0.10	0.010
3	0.50	0.050
4	5.0	0.50
5	10.	1.0

Table 4 Analytical results of various reference materials

	n	Mn, µg L^{-1} (^{71}Ga)[a]	As, µg L^{-1} (^{103}Rh)[a]	Cd, µg L^{-1} (^{103}Rh)[a]	W, µg L^{-1} (^{185}Re)[a]	Hg, µg L^{-1} (^{193}Ir)[a]	Pb, µg L^{-1} (^{193}Ir)[a]	U, µg L^{-1} (^{193}Ir)[a]
Seronorm™ Trace Elements Serum level 1								
Vial 1 Avg (SD)	7	14.8 (0.1)	0.657 (0.015)	0.131 (0.005)	0.109 (0.003)	0.791 (0.010)	1.11 (0.01)	0.052 (0.002)
Vial 2 Avg (SD)	6	14.9 (0.2)	0.773 (0.006)	0.129 (0.003)	0.112 (0.002)	0.791 (0.019)	1.11 (0.02)	0.053 (0.002)
Vial 3 Avg (SD)	7	14.9 (0.2)	0.541 (0.009)	0.149 (0.005)	0.086 (0.002)	0.785 (0.013)	1.21 (0.03)	0.067 (0.003)
Vial 4 Avg (SD)	7	14.9 (0.1)	0.737 (0.013)	0.134 (0.005)	0.110 (0.002)	0.795 (0.019)	1.10 (0.01)	0.048 (0.001)
Vial 5 Avg (SD)	8	14.8 (0.1)	0.935 (0.015)	0.134 (0.005)	0.134 (0.003)	0.787 (0.009)	1.13 (0.01)	0.053 (0.001)
Vial 6 Avg (SD)	8	14.8 (0.2)	0.736 (0.005)	0.132 (0.005)	0.114 (0.005)	0.785 (0.012)	1.11 (0.01)	0.051 (0.001)
Average (SE)[b]		14.8 (0.0)	0.730 (0.053)	0.135 (0.003)	0.111 (0.006)	0.789 (0.002)	1.13 (0.02)	0.054 (0.003)
CV, %[c]		0.4	17.9	5.5	13.7	0.5	3.8	12.1
Cert/Ref, µg L^{-1}		15.0±0.9	0.47	0.126	0.09	0.73±0.10	1.02	0.048
Avg Recovery, %		99	155	107	122	108	111	113
Seronorm™ Trace Elements Serum level 2								
Vial 1 Avg (SD)	3	19.7 (0.1)	0.544 (0.018)	0.137 (0.005)	0.082 (0.004)	1.62 (0.02)	1.39 (0.03)	0.056 (0.001)
Vial 2 Avg (SD)	7	19.7 (0.3)	0.904 (0.011)	0.132 (0.005)	0.118 (0.003)	1.62 (0.03)	1.36 (0.02)	0.063 (0.002)
Vial 3 Avg (SD)	7	19.8 (0.1)	0.372 (0.012)	0.132 (0.004)	0.065 (0.001)	1.62 (0.02)	1.35 (0.02)	0.081 (0.002)
Vial 4 Avg (SD)	8	19.8 (0.3)	0.775 (0.010)	0.137 (0.005)	0.109 (0.005)	1.63 (0.01)	1.35 (0.02)	0.079 (0.002)
Vial 5 Avg (SD)	8	19.9 (0.2)	0.852 (0.014)	0.138 (0.002)	0.120 (0.003)	1.65 (0.01)	1.38 (0.02)	0.090 (0.002)
Vial 6 Avg (SD)	8	20.0 (0.2)	0.929 (0.013)	0.133 (0.005)	0.148 (0.004)	1.65 (0.02)	1.37 (0.01)	0.061 (0.001)
Average (SE)[b]		19.8 (0.0)	0.730 (0.091)	0.135 (0.001)	0.107 (0.012)	1.63 (0.01)	1.37 (0.01)	0.072 (0.006)
CV, %[c]		0.5	30.6	2.1	27.6	0.9	1.1	18.8
Cert/Ref, µg L^{-1}		19.9±1.1	0.67	0.130	0.110	1.87±0.13	1.11	0.05
Avg Recovery, %		100	109	104	98	87	123	150
NIST Inorganic Constituents in Animal Serum - SRM 1598A								
Vial 1 Avg (SD)	4	2.08 (0.05)	0.339 (0.007)	0.063 (0.004)	0.051 (0.006)	0.028 (0.003)	1.43 (0.04)	0.022 (0.001)
Vial 2 Avg (SD)	9	2.11 (0.06)	0.347 (0.008)	0.073 (0.009)	0.053 (0.004)	0.028 (0.002)	1.44 (0.02)	0.023 (0.001)
Average		2.10	0.343	0.068	0.052	0.028	1.44	0.022
RPD, %[d]		1.3	2.3	15.1	3.7	1.3	0.7	4.2
Cert/Ref, µg L^{-1}		1.78±0.3	0.3	0.048±0.004		0.32±0.19		
Recovery, %		118	114	141		8.7		
INSPQ Serum PT								
Q1304 Avg (SD)	4	2.81 (0.07)	11.7 (0.3)	1.32 (0.03)	0.007 (0.005)	1.26 (0.03)	72.0 (1.5)	0.307 (0.012)
Target, µg L^{-1}		2.85	12.2	1.25		1.45	67.5	0.31
Recovery, %		99	96	106		87	107	100
Q1310 Avg (SD)	8	1.88 (0.01)	7.14 (0.15)	2.85 (0.04)	0.002 (0.001)	1.45 (0.06)	39.2 (0.6)	0.207 (0.005)
Target, µg L^{-1}		2.05	8.17	2.71		1.54	37.5	0.209
Recovery, %		91	87	105		95	104	99
Q1316 Avg (SD)	8	1.91 (0.03)	15.1 (0.3)	1.92 (0.02)	0.005 (0.002)	2.24 (0.12)	18.6 (0.2)	0.524 (0.010)
Target, µg L^{-1}		2.02	15.2	1.87		2.49	16.9	0.502
Recovery, %		95	99	103		90	110	104

[a] Internal standard used for the analysis.
[b] Standard error (SE) - standard deviation of six average values (vial 1 to 6) divided by the square root of the number of vials.
[c] CV, % - standard deviation of six average values (vial 1 to 6) divided by the average of six average values (vial 1 to 6).
[d] RPD, % - for only two values formally CV cannot be calculated.

Table 5 Statistical evaluation of in-house quality control human plasma reference materials

	Mn, µg L^{-1} (^{71}Ga)[a]	As, µg L^{-1} (^{103}Rh)[a]	Cd, µg L^{-1} (^{103}Rh)[a]	W, µg L^{-1} (^{185}Re)[a]	Hg, µg L^{-1} (^{193}Ir)[a]	Pb, µg L^{-1} (^{193}Ir)[a]	U, µg L^{-1} (^{193}Ir)[a]
MDL[b]	0.0155	0.00424	0.00237	0.00438	0.00676	0.00329	0.00207
Quality Control Reference Material – Level 1 (Low)							
n [c]	28	28	28	28	28	28	28
Mean	0.560	0.172	0.213	0.202	0.295	0.222	0.199
SD	0.015	0.005	0.004	0.004	0.007	0.007	0.004
CV, %	2.7	3.2	2.0	2.1	2.4	3.2	2.1
Quality Control Reference Material – Level 2 (High)							
n [c]	28	28	28	28	28	28	28
Mean	2.603	3.285	2.205	2.149	2.393	2.299	2.113
SD	0.043	0.050	0.033	0.033	0.057	0.036	0.035
CV, %	1.6	1.5	1.5	1.5	2.4	1.6	1.6

[a] Internal standard used for analysis.
[b] The MDL for each metal was determined separately using carefully selected human plasma specimens where concentration of the metal of interest was close to the expected method detection limit. Calculations are based on seven results (six degrees of freedom) acquired within a single analytical run. All MDLs refer to actual concentrations of the analytes in plasma, accounting for the plasma dilutions.
[c] The samples were analyzed between Oct 09, 2013 and Dec 03, 2013. All data were collected during 28 consecutive analytical runs at one measurement per day.