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Direct Speciation Analysis of Arsenic in Whole Blood and Blood Plasma at Low Exposure Levels by Hydride Generation-Cryotrapping-Inductively Coupled Plasma Mass Spectrometry

Tomáš Matoušek^{*,†}, Zhifeng Wang^{‡,§}, Christelle Douillet[‡], Stanislav Musil[†], and Miroslav Stýblo[‡]

[†]Institute of Analytical Chemistry of the Czech Academy of Sciences, v. v. i., Veveří 97, Brno 602 00, Czech Republic

[‡]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 2302 MHRC, Chapel Hill, North Carolina 27599-7461, United States

[§]School of Environmental Science and Engineering, Shandong University, 27 Shanda South Road, Jinan, Shandong 250100, China

Abstract

A method for analysis of toxicologically important arsenic species in blood plasma and whole blood by selective hydride generation with cryotrapping (HG-CT) coupled either to atomic absorption spectrometry (AAS) with a quartz multiatomizer or to inductively coupled plasma mass spectrometry (ICPMS) has been validated. Sample preparation, which involved only 5 times dilution with addition of Triton X-100, Antifoam B, and L-cysteine, suppressed excessive foaming in a hydride generator. Calibration slopes for whole blood and blood plasma spiked with arsenate, monomethylarsonate, and dimethylarsinate at 0.25–1 $\mu\text{g L}^{-1}$ As and 0.025–0.1 $\mu\text{g L}^{-1}$ As for AAS and ICPMS detection, respectively, did not differ from slopes in aqueous solutions. HG-CT-AAS was used to analyze samples with elevated levels of arsenic species-blood plasma from patients treated with arsenic trioxide for acute promyelocytic leukemia and whole blood from mice fed an arsenic-containing diet. A good agreement between results of the direct analysis and analysis after mild digestion in phosphoric acid proved the good efficiency of the direct HG-CT procedure for the arsenic species in these types of biological samples. In the next step, plasma and whole blood from healthy donors that were spiked with the plasma from leukemia patients at levels of 0.15–0.4 $\mu\text{g L}^{-1}$ As were analyzed by direct HG-CT-ICPMS. Good recoveries for all species even at these low levels (88–104%) were obtained. Limits of detection in blood and plasma were 0.014 $\mu\text{g L}^{-1}$ for inorganic arsenic and below 0.002 $\mu\text{g L}^{-1}$ As for methylated arsenic species. Thus, the

*Corresponding Author matousek@biomed.cas.cz.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.7b01868. Description of the experimental setup; scheme of the HG-CT-ICPMS system; description of the mild phosphoric acid digestion of blood plasma and whole blood (PDF)

Notes

The authors declare no competing financial interest.

ultrasensitive direct HG-CT-ICPMS method is uniquely suited for analyses of blood plasma and whole blood from individuals at low exposure levels.

Graphical Abstract

Arsenic is a toxic element present in body fluids in a number of species. From the point of arsenic toxicokinetics and metabolism, the most interesting species found in blood are inorganic arsenic (iAs, either arsenite, iAs^{III} , or arsenate, iAs^{V}) and their methyl derivatives, mono- (MAs) and dimethylated arsenic (DMAs), also in tri- and pentavalent forms. Compared to commonly analyzed urine samples, speciation analysis of arsenic in whole blood and blood plasma is no easy task. In a highly exposed population in Bangladesh, the total arsenic (tAs) concentrations in blood ranged from 1 to $80 \mu\text{g L}^{-1}$.¹ Thus, in a general, unexposed population, the levels of arsenic in whole blood are likely below $1 \mu\text{g L}^{-1}$ As, which is the limit of detection of the workhorse method of speciation analysis, liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (HPLC-ICPMS). Even lower arsenic concentrations (often below $0.1 \mu\text{g L}^{-1}$) were reported in blood plasma.²

In addition to arsenic concentrations being low, blood or plasma is also a difficult matrix, requiring either dilution or an extraction step prior to HPLC separation. Even analysis of tAs in blood or plasma at low exposure levels is very difficult.³ Arsenic species in blood are thought to be bound to proteins,⁴ which can also be a source of analytical artifacts.⁵ A recent review of clinical applications of arsenic speciation analysis⁶ lists only a few works on arsenic speciation analysis in whole blood, plasma, or serum. Typically, these reports only deal with samples with elevated arsenic contents, e.g., from high arsenic exposure areas,^{7–9} from patients undergoing arsenic trioxide treatment⁵ or after seafood or seaweed ingestion.^{10,11} For example, in a study examining arsenic species in maternal and cord blood, speciation analysis was successfully performed only in samples with tAs over $10 \mu\text{g L}^{-1}$.⁷ Similarly, Ito et al.¹² analyzed 16 pairs of urine/blood samples where concentration in urine exceeded $50 \mu\text{g L}^{-1}$; in all but one blood sample, only arsenobetaine (AsB) was present above the limit of detection (LOD) of $0.3 \mu\text{g L}^{-1}$.¹² Todorov et al. performed speciation analysis in several blood reference materials containing rather high levels of certified tAs ($14\text{--}177 \mu\text{g L}^{-1}$).¹³ Also, authors of an interlaboratory study of speciation analysis of

caprine blood spiked with individual arsenic species at levels of tAs 3–10 $\mu\text{g L}^{-1}$ declared that “reasonable results” were obtained considering that the concentrations of arsenic species were close to the measurement LODs of participant laboratories.¹⁴ This also illustrates the problem of reference materials suitable for method validation. Although there is plenty of blood, plasma, and serum reference materials (SRMs) on the market, only tAs is certified at levels 1 order of magnitude higher than those typically associated with low exposure, and most of these SRMs use a matrix spiked with arsenic species, as the levels of naturally occurring species are too low. The closest to a low arsenic SRM is NIST 955c Toxic Metals in Caprine Blood Level 1, noncertified reference value of $2.07 \pm 0.67 \mu\text{g L}^{-1}$ tAs (although there have been discrepancies in the interlaboratory study leading to an information value of $<5 \mu\text{g L}^{-1}$ tAs).¹⁵ Another low-level SRM is SERO201405, Seronorm Trace Elements in Serum, Level 1, with an approximate value of $0.47 \mu\text{g L}^{-1}$ tAs. To the best of our knowledge, there is currently no suitable reference material for blood, plasma, or serum with arsenic speciation information.

Our laboratories have recently developed a method based on selective hydride generation with cryotrapping (HG-CT) and atomic absorption spectrometry (AAS). This approach allows one to perform the analysis with a minimum sample pretreatment, as has been previously validated for a number of complex biological matrices: urine, cell lysates, arsenic enzymatic methylating assays, and even homogenates of liver tissue.^{16–20} Samples are not extracted but either simply lysed and diluted by deionized water for selective determination of trivalent species and trimethylarsine oxide or prereduced by addition of L-cysteine for measurement of sums of tri- and pentavalent species. This minimally pretreated sample is directly introduced into the hydride generator as a suspension. A connection of the identical HG-CT system to a more sensitive ICPMS detector allowed the LODs to decrease to low ng L^{-1} and sub ng L^{-1} levels for iAs and methylated species, respectively, permitting, e.g., the analyses of microsamples of exfoliated urothelial cells.^{21,22} HG-CT-AAS was also proven to be less prone to analytical artifacts in the analysis of arsenic species in methylating enzyme assays and urine than ion-pairing HPLC-ICPMS.²⁰

The aim of this work was to adapt and validate the HG-CT-ICPMS procedure for speciation analysis of arsenic in whole blood and blood plasma at the levels of low arsenic exposure. The issue of sample foaming had to be addressed.

EXPERIMENTAL SECTION

Arsenic Standards and Reagents.

The following arsenicals were used for preparation of stock standard solutions at concentrations of $100 \mu\text{g L}^{-1}$ by serial dilution in deionized water (DIW). Disodium hydrogen arsenate (Na_2HAsO_4) (99% pure) was purchased from Sigma-Aldrich (St. Louis, MO). Methylarsonic acid, disodium salt ($\text{CH}_3\text{AsO}(\text{ONa})_2$), and dimethylarsinic acid ($(\text{CH}_3)_2\text{AsO}(\text{OH})$) both better than 98% pure were purchased from Chem Service (West Chester, PA). The absence of other arsenic species in these standards was tested, and tAs content in each of these standards was determined by graphite furnace-AAS.¹⁶ Mixed working standards of iAs, MAs, and DMAs at appropriate concentrations were prepared fresh daily.

HG was performed from 0.75 M Tris(hydroxymethyl)-aminomethane hydrochloride buffer (TRIZMA hydrochloride, Sigma-Aldrich; Tris) adjusted to pH 6 by KOH. Reductant solution was 1% NaBH₄ (Fluka) in 0.1% KOH and 0.1% Antifoam B (Sigma-Aldrich). Antifoam B and Triton X-100 (Sigma-Aldrich, Triton) were also added to the plasma and blood samples. For prereduction of pentavalent arsenic species, L-cysteine hydrochloride monohydrate (for biochemistry grade, Merck; L-cys) was used throughout.^{16–20}

HG-CT-AAS and HG-CT-ICPMS Setup.

The experimental arrangement for HG-CT-AAS speciation analysis was essentially equivalent to that described in our previous reports.^{18,19} The AAS spectrometer (AAnalyst 800, PerkinElmer) was operated with an arsenic electrodeless discharge lamp (System II, PerkinElmer) and with a deuterium background correction. For atomization, a quartz multiatomizer¹⁶ heated to 900 °C with an outer air flow rate of 35 mL min^{−1} was used.

Interfacing of HG-CT to the ICPMS detector was described in ref 21. The ICPMS spectrometer (Agilent 7500x) was operated in a collision mode (He 3.9 mL min^{−1}). A thorough description of the experimental setup including a system scheme and procedure is presented in the Supporting Information.

Both AAS and ICPMS time-resolved data were exported into MS Excel files for integration. Peak areas were used exclusively for quantification. The ICPMS peak area data were corrected according to average ¹²⁵Te internal standard signal over the peak integration interval if significant sensitivity drifts were observed. Concentrations of arsenic species are shown as averages; the number in parentheses shows a standard deviation of a total of two parallels with two replicates each for analysis after digestion and three replicates each for direct HG-CT-AAS/ICPMS, respectively.

Blood and Plasma Samples.

Sodium heparin stabilized whole human blood (pooled) was purchased from BioreclamationIVT (Westbury, NY) and shipped on ice. Samples were stored at 8 °C and analyzed within 1 week from delivery. Blood plasma was obtained by centrifugation of this blood for 10 min at 800 rpm and 4 °C.

Human plasma samples with elevated arsenic levels were collected originally for a clinical study “A Pharmacologic Study of Arsenic Trioxide in Cancer Patients” led by Dr. Cristina Ghiuzeli, North Shore University Hospital, and Dr. Miroslav Stýblo, University of North Carolina at Chapel Hill. The goal of this study was to characterize the metabolism of arsenic trioxide (ATO) and kinetics of ATO and its metabolites in plasma of promyelocytic leukemia patients treated with ATO. In general, the samples were collected before and starting 1 h after ATO infusion and then daily for several days. The actual plasma samples used in the presented study were selected from these sets to contain suitable amounts of all arsenic species according to previous analyses. They were collected at day 9 of the ATO therapy and stored at −80 °C for approximately 6 months.

Murine whole blood samples with elevated arsenic levels originated from a pilot study that compared metabolism of iAs^{III} in wild-type mice and mice with null genotype for GST-P,

GST-M, and GST-T, the three isoforms of glutathione S-transferase. This mouse strain was created by Dr. Beverly Koller at UNC Chapel Hill.²³ The mice drank deionized water and were fed a regular (grain based) laboratory diet in which iAs levels were found to range from 89 to 139 $\mu\text{g kg}^{-1}$.²⁴ Male mice were used and were not fasted before blood collection. Blood was collected in a heparin capillary from submandibular bleed. To provide sufficient volume of sample, blood was pooled from 4 mice into tubes containing sodium fluoride and disodium EDTA (BD 365992) and stored at 4 °C until analysis. Samples were analyzed within 2 days from collection. All procedures involving mice were approved by the University of North Carolina (UNC) Institutional Animal Care and Use Committee.

For direct analysis, 160 μL of whole blood or plasma was mixed with 40 μL of 10% Antifoam B and 80 μL of 10% Triton solutions, standard additions if applicable, and diluted by DIW to total volume of 720 μL . At least 1 h prior to analysis, 80 μL of 20% L-cys solution was added to the sample mixture. Standards for calibration were prepared by the same procedure with addition of Antifoam B and Triton and prereduced by L- cys. Samples were prepared in 2 parallels and 3 replicates of each sample were measured. 250 μL of mixture was sampled into HG-CT, followed by two 300 μL DIW rinses. The description of the procedure for mild digestion in ultrapure phosphoric acid¹⁹ for comparative analyses is provided in the Supporting Information.

RESULTS AND DISCUSSION

Mixture Foaming Treatment.

The inherent advantage of a HG-CT technique is the separation of analytes from a liquid matrix or a suspension. Once the arsenic species from the samples are converted to corresponding arsanes, released into the gaseous phase, and further “purified” in the NaOH dryer cartridge, the steps of cryotrapping/separation and detection are practically matrix independent. The research therefore focused on the step of generation of arsanes from the matrix of interest.

A cardinal problem of direct HG from both blood plasma and whole blood sample matrix was formation of thick and persistent foam in the gas–liquid separator. Capturing of gaseous analyte hydrides within the foam caused lower recoveries. Pronounced memory effects were also observed. Simple addition of Antifoam B agent into the reductant solution, proven efficient for direct analysis of urine, cell lysates, and tissue slurries, was not sufficient for this matrix. Addition of Antifoam B into the sample did not improve the situation. However, addition of surfactant Triton along with Antifoam B into the sample changed the foam characteristics. The foam decomposed very quickly and completely disappeared within 1 min after the end of HG. Moreover, Triton is also often used to facilitate lysing of the cells, which may improve the availability of arsenic from the cells to HG reaction.

In pilot experiments, sample dilution and the optimum Antifoam B and Triton concentrations were tested for optimum performance, both for visual foam production and recovery of species spiked into the plasma at 1 $\mu\text{g L}^{-1}$ level with the AAS detection. The best composition of sample mixture was found to be as follows: 160 μL or less of plasma or blood per 800 μL of mixture containing 1% Triton, 0.5% Antifoam B, and 2% L- cys for

prereduction (dilution factor of sample 5 or higher), while reductant solution also contained 0.1% of Antifoam B. Higher concentrations of Triton or Antifoam did not increase the signals of arsenic species. Other HG parameters were left unchanged as optimized in previous studies,^{16,19,21} since the sensitivities of arsenic species in the presence of blood and plasma matrix were the same as in aqueous solutions, as shown below.

250 μL of sample mixture (corresponding to 50 μL of original blood or plasma per replicate) was pipetted into the system, followed by 2 times of a 300 μL rinse of DIW to flush the sample quantitatively into the system and to reduce carryover between the samples. This is a compromise between the maximum sensitivity and typical sample volumes available in planned studies, allowing 3 replicate measurements from 160 μL of sample.

HG-CT-AAS.

At these conditions, the HG-CT system with the AAS detection was used to verify the performance of the hydride generation, especially the efficiency of production of volatile arsanes in the presence of such a difficult matrix. Slopes of calibration graphs of aqueous solutions with Triton and Antifoam B at concentrations of 0.25–1.5 $\mu\text{g L}^{-1}$ of iAs, MAs, and DMAs were compared with the slopes in blood plasma and whole blood spiked at the same concentrations in the HG-CT-AAS system. The slopes were $87.9 \pm 2.1\%$, $91.1 \pm 1.8\%$, and $93.1 \pm 1.7\%$ for iAs, MAs, and DMAs, respectively, in blood plasma and $97.6 \pm 2.5\%$, $95.2 \pm 2.4\%$, and $92.9 \pm 1.9\%$ for iAs, MAs, and DMAs, respectively, in whole blood. The slopes of MAs and DMAs were within 96–107% from the iAs slope both in aqueous, plasma, and blood matrices. Notably, the slopes (0.995 s ng^{-1} for iAs) exhibit excellent day to day repeatability, and they correspond well among different laboratories using multiatomizers of the same dimensions and experimental parameters with similar systems.^{16,17,25} Method LODs with dilution factor of 5 are detailed in Table 1.

Generally, in blood, arsenic species are thought to be bound to proteins. Therefore, spiking of samples by simple standards of these species may not be sufficient for proper method validation. To verify the accuracy of the direct HG-CT-AAS speciation analysis in blood plasma, analysis of two samples of plasmas of leukemia patients was performed. Samples chosen for the validation contained 12–35 $\mu\text{g L}^{-1}$ of each arsenic species, presumably in the forms of the naturally occurring metabolites. These concentrations are high enough to allow HG-CT-AAS analysis after phosphoric acid digestion which preserves the information about the methylation status of arsenic species.¹⁹ Method LODs after digestion considering a dilution factor of 14 are displayed in Table 1. The higher LOD for iAs was caused by elevated blank values (approximately $0.11 \mu\text{g L}^{-1}$ iAs) from phosphoric acid, even though a very high purity reagent was used. The comparison of found values is in Table 2. Results of the direct HG-CT-AAS are in good agreement with the analysis after mild digestion; the recoveries of individual arsenic species are between 89% and 114%. An analysis of tAs content for the validation purposes was not performed, because blood and plasma samples can possibly contain a significant fraction of AsB,^{9,11,12} which is not hydride active, and therefore, the sum of species would not be complete. Furthermore, AsB is considered harmless with no significance from the toxicological point of view as compared to the arsenic species investigated.

While the above experiment demonstrated accurate analysis of arsenic species present in plasma, it does not verify the analysis of arsenic species associated with blood cells. Unfortunately, whole blood samples from the leukemic study or other groups of individuals exposed to arsenic were not available. Therefore, whole blood samples of arsenic exposed mice currently obtainable from an ongoing metabolic study were used instead. Two whole blood samples were analyzed both by direct HG-CT-AAS and by HG-CT-AAS after mild digestion in phosphoric acid. The concentrations of iAs in these two samples were 2.43 (0.14) and 2.72 (0.16) $\mu\text{g L}^{-1}$ As after digestion compared to 2.40 (0.03) and 2.81 (0.08) $\mu\text{g L}^{-1}$ As by direct HG-CT-AAS, and for DMAs, it was 1.06 (0.12) and 0.67 (0.13) $\mu\text{g L}^{-1}$ As after digestion compared to 0.88 (0.03) and 0.72 (0.07) $\mu\text{g L}^{-1}$ As by direct HG-CT-AAS. The MAs values were under the LOD of both methods. Considering the fact that the levels were very close to the limit of quantification (LOQ) of the digestion method (Table 1), there is good agreement of results, proving efficient direct generation of volatile arsanes even from species associated with blood cells.

HG-CT-ICPMS.

The ICPMS detector can be easily coupled with an essentially identical HG-CT system providing excellent sensitivity and LODs.²¹ Therefore, the performance of such a system was tested for samples of blood plasma and whole blood.

Similarly to analyses using the AAS detector, slopes of iAs, MAs, and DMAs calibration graphs in aqueous solutions and in plasma and whole blood were compared. With the ICPMS detector, the spiking was performed at ten times lower levels than for AAS, i.e., at 0.025, 0.05, and 0.1 $\mu\text{g L}^{-1}$ arsenic species in the sample mixture. In this case, the slopes related to aqueous calibration were $95.0 \pm 2.8\%$, $95.3 \pm 1.2\%$, and $99.8 \pm 1.0\%$ for iAs, MAs, and DMAs, respectively, in blood plasma and $97.4 \pm 3.1\%$, $94.7 \pm 1.4\%$, and $94.6 \pm 1.3\%$ for iAs, MAs, and DMAs, respectively, in whole blood. Slopes related to iAs were 97–94% and 92–85% for MAs and DMAs, respectively. The levels found in our test matrix sample, i.e., pooled human blood, were as follows: 0.088 (0.005), 0.041 (0.003), and 0.029 (0.001) $\mu\text{g L}^{-1}$ iAs, MAs, and DMAs, respectively, in the whole blood, and 0.047 (0.005), 0.017 (0.0004), and 0.024 (0.001) $\mu\text{g L}^{-1}$ iAs, MAs, and DMAs, respectively, in plasma obtained from that blood. This totals to 0.158(0.006) $\mu\text{g L}^{-1}$ As in whole blood and 0.088 (0.005) $\mu\text{g L}^{-1}$ As in plasma. It should be noted that this sum of arsenic species does not include any species undetectable by hydride generation methods, such as AsB which could be present in blood.

In Figure 1, chromatograms of blank, mixed standard, and unspiked whole blood sample are shown. The fourth peak in the chromatogram corresponds to trimethylarsane which is consistently present in blanks at levels of approximately 0.001–0.002 $\mu\text{g L}^{-1}$.^{21,26} LOD and LOQ values are provided in Table 1. These limits are controlled by achievable reagent blanks, especially for iAs, when the blank was approximately 0.008–0.016 $\mu\text{g L}^{-1}$, comparable to previous works with a similar system.²¹ No single reagent could be pinpointed as a clear source of elevated iAs blanks, except L-cys, for which using various sources and batches resulted in different blank values.

The precision expressed as relative standard deviation of measurements in plasma and whole blood matrix was always better than 5% and typically better than 3% in the range of 0.01–0.1 $\mu\text{g L}^{-1}$ ($n = 6$, 2 aliquots 3 replicates each). Blood plasma and whole blood samples were also spiked with leukemic plasmas containing naturally occurring arsenic metabolites previously analyzed by HG-CT-AAS (see above) and tested for recovery of arsenic species. Spiking was performed at the levels consistent with very low arsenic exposure (0.15–0.4 $\mu\text{g L}^{-1}$, i.e., 0.03–0.08 $\mu\text{g L}^{-1}$ after dilution). The results of recovered leukemic plasma concentrations (Table 2) confirm good recoveries for all species even at these low levels: 88–104% in plasma and 91–99% in whole blood (related to results by HG-CT-AAS after phosphoric acid digestion).

CONCLUSION

A method for analysis of arsenic species in blood plasma and whole blood was proven sufficiently sensitive and accurate at levels corresponding to a low arsenic exposure. This HG-CT-ICPMS based method is an excellent complement of currently used HPLC-ICPMS methods, which it surpasses especially in LODs and in the minimum sample pretreatment requirements. This method would be capable of speciation analysis even for control, unexposed groups in the population or laboratory studies. On the other hand, although this method can provide quantification of the most toxicologically interesting species, it cannot provide complete arsenic speciation.

The method is based on a technique validated also for the oxidation state specific analysis of inorganic and methylated arsenic species in a number of biological matrices including tissue slurries.^{16,19} Here, we limited the validation to sums of tri- and pentavalent species. The validation of oxidation state specific analysis was beyond the scope of this work, as this would require also the study of stability of trivalent methylated species and proper storage of the human and mouse samples.

Although our HG-CT method uses the ICPMS detector to achieve sufficient sensitivity for low exposure levels, it can be with minimum modification used with a simpler atomic fluorescence detector with similar LODs.²⁶ From the practical point, the application of a semiautomated version of the cryotrapping procedure¹⁶ as well as standardization on a single arsenic species for routine analyses are straightforward. Moreover, the addition of surfactant along with Antifoam B to the sample may be useful also for other types of samples producing excessive foam formation during hydride generation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Typical signals obtained with HG-CT-ICPMS. Dotted line: blank; dashed line: mixed standard of iAs, MAs, and DMAs ($0.025 \mu\text{g L}^{-1}$ As each); solid line: unspiked whole blood. 1: iAs; 2: MAs; 3: DMAs; 4: trimethylarsane.

Table 1.

Limits of Detection and Quantification in Blood Plasma and Whole Blood for Individual Methods

method	dilution factor		iAs, $\mu\text{g L}^{-1}$	MAs, $\mu\text{g L}^{-1}$	DMAs, $\mu\text{g L}^{-1}$
phosphoric acid digestion HG-CT-AAS	14	LOD	0.6	0.2	0.2
		LOQ	2.1	0.7	0.6
direct HG-CT-AAS	5	LOD	0.15	0.09	0.07
		LOQ	0.49	0.29	0.25
direct HG-CT-ICPMS	5	LOD	0.014	0.002	0.001
		LOQ	0.047	0.008	0.004

Table 2.

Concentrations of Arsenic Species in Leukemic Plasma Samples

method	dilution factor	iAs, $\mu\text{g L}^{-1}$	MAs, $\mu\text{g L}^{-1}$	DMAs, $\mu\text{g L}^{-1}$
Leukemic Plasma 1				
phosphoric acid digestion HG-CT-AAS	14	16.0 (0.4)	31.7 (0.4)	33.4 (0.6)
direct HG-CT-AAS	11	14.2 (0.4)	29.7 (0.5)	37.2 (0.5)
HG-CT-ICPMS (spiked into blood plasma)	528	16.4 (0.3)	30.6 (0.7)	34.8 (0.7)
HG-CT-ICPMS (spiked into whole blood)	522	15.8 (0.6)	29.6 (1.1)	32.6 (0.6)
Leukemic Plasma 2				
phosphoric acid digestion HG-CT-AAS	14	19.4 (0.7)	15.3 (0.7)	13.7 (0.4)
direct HG-CT-AAS	5	19.6 (0.7)	14.2 (0.5)	15.5 (0.6)
HG-CT-ICPMS (spiked into blood plasma)	268	19.2 (0.3)	13.5 (0.3)	14.1 (0.3)
HG-CT-ICPMS (spiked into whole blood)	232	18.8 (0.6)	14.0 (0.5)	13.4 (0.3)