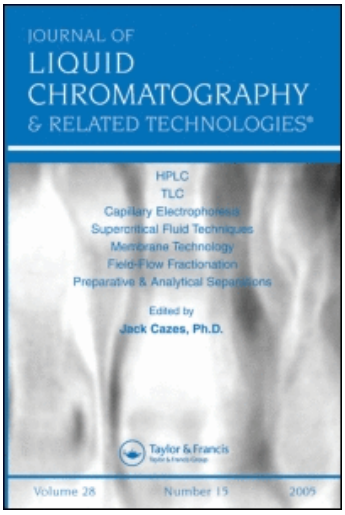
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Trace Analysis and Speciation for Arsenic Anions by HPLC-Hydride Generation Inductively Coupled Plasma Emission Spectroscopy

D. S. Bushee[a]; I. S. Krull[a]; P. R. Demko[b]; S. B. Smith Jr.[b]
[a] The Barnett Institute of Chemical Analysis and Materials Science Department of Chemistry, Northeastern University, Boston, Massachusetts, USA [b] Analytical Instrument Division, Instrumentation Laboratory, Inc., Andover, Massachusetts, USA

To cite this Article Bushee, D. S. , Krull, I. S. , Demko, P. R. and Smith Jr., S. B.(1984) 'Trace Analysis and Speciation for Arsenic Anions by HPLC-Hydride Generation Inductively Coupled Plasma Emission Spectroscopy', Journal of Liquid Chromatography & Related Technologies, 7: 5, 861 — 876
To link to this Article: DOI: 10.1080/01483918408074008
URL: http://dx.doi.org/10.1080/01483918408074008

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(5), 861-876 (1984)

TRACE ANALYSIS AND SPECIATION FOR ARSENIC ANIONS BY HPLC-HYDRIDE GENERATION-INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY

D.S. Bushee* and I.S. Krull*
The Barnett Institute of Chemical Analysis and Materials Science
Department of Chemistry
Northeastern University
360 Huntington Avenue
Boston, Massachusetts 02115 USA

and

P.R. Demko and S.B. Smith, Jr.
Analytical Instrument Division
Instrumentation Laboratory, Inc.
1 Burtt Road
Andover, Massachusetts 01810 USA

Downloaded At: 17:09 24 January 2011

ABSTRACT (26)

High performance liquid chromatography (HPLC) has already been successfully interfaced with inductively coupled plasma (ICP) emission spectroscopy for arsenic analysis and speciation. However, in many instances the overall minimum detection limits (MDLs) are inadequate for many environmental type samples. Arsine generation in a continuous, on-line fashion has been shown to provide for significantly improved MDLs by direct-ICP approaches. This hydride generation-ICP (HY-ICP) derivatization approach has now been successfully interfaced with paired-ion, reversed phase HPLC. This provides a doubly hyphenated technique, namely HPLC-HY-ICP in order to perform true metal/non-metal speciation. Such methods of arsenic speciation have now been perfected with regard to minimum detection limits, linearity responses over several orders of magnitude, separation of various arsenic species from possible sample interferences, and related analytical matters. The final approaches have been applied both to spiked water samples, as well as to actual environmental drinking water supplies from the New England region. These results demonstrate an ability to qualitatively and quantitatively speciate for arsenate and arsenite at levels ranging from 50 ppb and above in each species. The ability to speciate drinking water supplies (wells) is also demonstrated by these overall application results.

*Authors to whom correspondence and reprint requests should be addressed.

Copyright © 1984 by Marcel Dekker, Inc. 0148-3919/84/0705-0861$3.50/0

INTRODUCTION (26)

Within recent years interest has grown concerning the development of newer analytical methods for the successful and accurate speciation of metal or nonmetal derivatives or species (1-5). This has occurred because of the growing realization that different forms or species of a metal can exert different toxicological and biological properties in animal and human systems (6-9). Thus, the belief that toxicological observations correlate with total metal content has now fallen into some disrepute. Many published toxicological studies that attempt to relate total metal analysis or content to observed toxicological properties must therefore be reconsidered in light of the more recent suggestions that individual metal or nonmetal species can have very different biological effects in man. Total metal content in place of metal or nonmetal speciation type analyses tells us very little about what toxic properties are really due to individual metal or nonmetal species present. In order to provide valid toxicological results and interpretations, one must therefore have valid analytical speciation methods for just those metal or nonmetal species present in any given sample. This is not always an easy or straight-forward analytical task. In addition, many toxicologists do not have the background to undertake speciation analyses along with their desired toxicological studies. There has thus evolved an extensive metal and non-metal toxicology literature which involves little real inorganic speciation data. This current state of affairs can only be corrected when the analytical chemist develops suitable trace methods of analysis and speciation for many of those metal or nonmetal species of interest to environmentalists and toxicologists or biologists.

Downloaded At: 17:09 24 January 2011

Arsenic has long been known to have severe toxic properties in mammalian systems, but much less is known with regard to the toxic properties of various oxyanion derivatives. The trivalent forms of arsenic are more toxic than the pentavalent forms. At a level of 10 ug/g (ppm), sodium arsenite has produced embryotoxicity and teratogenic effects in mice, while at the same level, sodium arsenate had no obvious effect (7). Epidemiologic studies have suggested that arsenic in drinking water may be related to an increased incidence of skin cancer. Arsenate is the valence form most prevalent in nature, and in this form, it tends to be rapidly excreted and probably does not accumulate. Arsenite is the trivalent species ($NaAsO_2$), while arsenate is the pentavalent form (Na_2HAsO_4). All of the arsenic species of interest exist mainly as oxyanions, with valences usually of -1 or -2. Within recent years, a problem has appeared within various New England states, such as New Hampshire, wherein high levels of total arsenic have been found in certain drinking water supplies. Apparently lead arsenate has been used as an agricultural

chemical and pesticide in various fruit orchards in New England. Residues of this arsenic derivative have gradually leached from the orchards into the soil, and from there into various wells used for drinking water. Total levels of arsenic have been found as high as 200 ppb to 250 ppb (parts-per-billion), but they are more commonly 100 ppb or below. Methods for conveniently and reliably performing arsenic speciation of such drinking water supplies have not, in general, been applied to well water samples from New England or elsewhere.

We, as well as others, have demonstrated the capabilities of paired-ion, reversed phase HPLC (RP-HPLC) for performing metal anion analyses, with an emphasis on speciating various arsenic oxyanions (1, 3, 10-17). Conventional ion exchange HPLC or ion chromatography are also fully capable of resolving the various arsenic oxyanions, and such approaches have already been interfaced with inductively coupled plasma (ICP) emission spectroscopy, graphite furance atomic absorption (GFAA) spectroscopy, flame atomic absorption (FAA) spectroscopy, direct current plasma (DCP) emission spectroscopy, electrochemical detection (EC), conductivity detection (CD), and similar specific or nonspecific HPLC detection methods (1). Earlier, we had described an HPLC-ICP approach for speciating at least three different arsenic derivatives (3). These methods of speciation, at least in our hands, did not provide useful and practical minimum detection limits (MDLs). This precluded their direct application to environmental samples, such as well water from New Hampshire.

It occurred to us that there were at least two approaches for immediately improving (lowering) the MDLs with HPLC-ICP, especially for arsenic speciation purposes. One of these might have involved an initial sample preconcentration step using electrothermal carbon cup vaporization of the HPLC eluents just prior to ICP sample introduction (23). Indeed, this particular approach has recently been described at some length by Caruso *et al*. (24). We have always felt that this approach, though general for virtually all metals or nonmetals and capable of providing, in principle, usable MDLs, might at the same time have certain difficult-to-achieve interfacing problems for continuous HPLC-ICP operation. The recent report by Caruso *et al*. that has used electrothermal carbon cup vaporization of the individual HPLC analytes for ICP detection operated in an entirely off-line manner. We have believed that a successful tandem analytical approach should be able to operate on a continuous, on-line basis, with as little operator intervention as possible. Indeed, it should also be able to analyze a large number of environmental samples in an automated fashion, similar to HPLC-ultraviolet (UV) detection or liquid chromatography-electrochemical detection (LCEC).

Another possible solution to the problem of suitable MDLs in HPLC-ICP applications appeared to reside in the use of continuous hydride formation or

Downloaded At: 17:09 24 January 2011

generation post-column, with efficient introduction of such metal or nonmetal hydrides into the ICP. Though this particular approach had never been used with HPLC-ICP interfacing, the formation of arsine from various arsenic derivatives has long been known. This approach has been used to improve MDLs for FAA and ICP (18, 19, 25). It therefore occurred to us, as it has to others, that hydride generation (HY) after the HPLC separation step, just before the ICP detection step, might vastly improve overall MDLs for the final speciation of arsenicals (20, 21). This same approach should just as readily be applicable to all other metal or nonmetal species capable of forming hydrides by suitable reaction with sodium borohydride or other reagents. We have, as yet, only applied HPLC-HY-ICP for certain arsenic species, with excellent overall analytical results. The approach provides MDLs of about 50 ppb for environmental type samples or artificially spiked water samples, with a high degree of accuracy and precision. The methods have been applied to a number of artificially spiked water samples for both arsenate and arsenite. In addition, they have been used for a number of New Hampshire well water samples, using both HPLC-HY-ICP for speciation purposes and direct HY-ICP for total arsenic determinations.

Downloaded At: 17:09 24 January 2011

EXPERIMENTAL

Reagents

Sodium arsenite, sodium arsenate and sodium hydroxide were Baker Analyzed, reagent grade chemicals from VWR Scientific, Inc. (Boston, Mass.). Sodium dimethylarsenate (SDMA) was obtained from Pfaltz and Bauer, Inc. (Stamford, Conn.). External arsenic standards for Direct-HY-ICP were Baker Analyzed. Mobile phase water for HPLC was taken from a custom made still by Barnstead Co., Division of Sybron, Inc. (Boston, Mass.). Sodium borohydride, 98% $NaBH_4$, was obtained from Alfa Products, Thiokol Corporation, Ventron Division (Danvers, Mass.). Mobile phase and sodium borohydride solutions were filtered through 0.45 um filters (Gelman Sciences, Inc., Ann Arbor, Mich.). Hydrochloric acid (HCl) was of Baker high purity grade Ultrex acids.

Apparatus

This work was performed using an Instrumentation Laboratory (IL) Model Plasma-200 (Instrumentation Laboratory, Inc., Andover, Mass.). A standard cross flow nebulizer spray chamber with a modified drain trap was used to introduce the HPLC eluent to the plasma region. The drain trap was positioned directly beneath the spray chamber. Waste flowed directly into the covered drain trap reservoir by means of a conical plastic attachment. The HPLC system consisted of a Laboratory Data Control (LDC) (Rivier Beach, Fla.) Constametric I pump,

a Rheodyne Model 7125 syringe injection valve (Rheodyne Corp., Cotati, Calif.) fitted with a 200 ul loop and a Honeywell Corp. (Minn., Minn.) dual pen strip chart recorder. Data reduction of the ICP emission intensity data was performed with a Radio Shack TRS-80 Model II microcomputer (Tandy Corp., Fort Worth, Texas). The computer was used to calculate peak areas from raw ICP emission data.

HPLC separations were performed on a number of commercial C_{18} columns as follows: 1) Excalibar Spherisorb ODS (5 um, pre-packed column, 4.6-mm x 15-cm)(Applied Science, State College, Penna.); 2) an in-house slurry packed (4.6-mm x 25-cm) column using 10 um Lichrosorb C_{18} (MCB Chemicals, Inc., Cinc., Ohio); 3) an in-house slurry packed column (4.6-mm x 15-cm) using 5 um Ultrasphere ODS (Altex/Beckman, Berkeley, Calif.); or 4) a pre-packed 10 um High Performance (HP) guard column (10-cm x 3.2-mm)(Alltech Assocs., Deerfield, Ill.).

The hydride generator was constructed from two glass tees (Technicon Corp., Tarrytown, N.Y.), part no. 116-0200-045, connected with 1/16" Teflon tubing running from the end of the HPLC column to the ICP nebulizer. Reagent solutions were introduced with a dual channel peristaltic pump, Figure 1, which was home-made. Direct HY-ICP was done using the IL Plasma Hydride Device.

Downloaded At: 17:09 24 January 2011

Methods

The HPLC mobile phase consisted of 5 mM PIC A (tetrabutylammonium phosphate reagent (Waters Associates, Milford, Mass.) in distilled water, prepared according to the manufacturer's directions with a final pH of 7.15. Columns were operated at room temperature at a flow rate of normally 1 ml/min. Each column was washed at the end of each working day with 50:50 MeOH:HOH, to ensure complete removal of all ion-pairing reagent and salts.

Solutions of $NaBH_4$ were prepared in 0.25% sodium hydroxide (NaOH). Three to four percent solutions were made for HPLC-HY-ICP and filtered under vacuum. The plasma would become unstable at more concentrated solutions due to the increase in hydrogen gas formed. At lower $NaBH_4$ concentrations the overall arsine conversion efficiency would decrease. Concentrated HCl was used as received, and introduced separate from the other reagents, all at the same flow rate of 0.25 ml/min. Arsenic standards for HPLC were prepared fresh each day by dissolving the inorganic salts in distilled water, as used for the mobile phase. Injection volumes of 200 ul were used for all blanks, standards, spiked water and environmental well water samples.

Direct-Hydride Generation-ICP was done using the IL Plasma Hydride Device (PHD) with a 2% solution of $NaBH_4$. Samples were adjusted to 3M HCl just prior to analysis. External standards for Direct-HY-ICP were prepared by

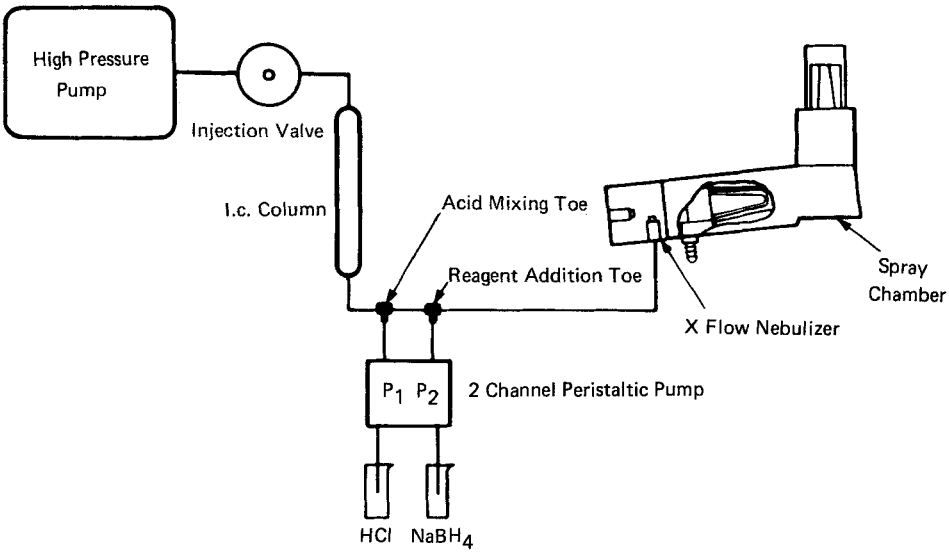


Figure 1. Schematic diagram of the total HPLC-HY-ICP instrumentation.

Downloaded At: 17:09 24 January 2011

appropriate dilution of 1000 ppm solutions of Baker Analyzed arsenic standard in 3M HCl.

Well water samples were collected from New Hampshire in glass jars with no acid or preservatives added and were refrigerated on receipt. No sample preparation was involved prior to HPLC-HY-ICP analysis. A minimum of three (3) injections were made of each sample and blank injections were made both before and after all sample injections.

The ICP operating parameters used throughout these studies with both Direct-HY-ICP and HPLC-HY-ICP were as follows: forward power (watts) = 1,000; bandpass (nm) = 0.02; observation height (mm) = 14; gas flows: coolant = 15 l/min; auxillary = 5 l/min; sample = 1 l/min. The 228.81 nm emission line for arsenic was used throughout all analyses.

Arsine conversion efficiences were determined for each arsenic species by measuring peak heights or emission intensity readings as a function of systematically varying the hydride generation conditions, including reagent concentrations, flow rates of reagents, mixing tees, contact times, and similar reaction parameters. It is believed that this experimental approach led to maximization of arsine formation and introduction into the ICP plasma, but thus far this is still an assumption. We have not yet demonstrated a 100% efficiency for the formation of arsine from any of these arsenic species, but

in principle there is at least one approach to demonstrate this experimentally. Studies are now underway in order to fully demonstrate the actual efficiencies of hydride generation as a function of the individual arsenic species. Despite the current assumption of maximum hydride formation, rather than absolute demonstration, our final MDLs for Direct-HY-ICP and HPLC-HY-ICP are adequate for practical environmental sample applications, as below.

RESULTS AND DISCUSSION

Minimum Detection Limits (MDLs) and Linearity of Calibration Plots

Figure 1 illustrates the overall HPLC-HY-ICP experimental apparatus, wherein the hydrides are formed in the Plasma Hydride Device after the HPLC separation. The hydride (arsine) together with the HPLC mobile phase and excess hydride generation reagents are all introduced into the conventional ICP cross-flow nebulizer. This acts as a gas-liquid separator, wherein most or all of the arsine is transferred to the ICP plume, but most (95-99%) of the aqueous solution eventually ends up not entering the same plume region. The cross flow nebulizer acts in HPLC-HY-ICP just as it does in conventional Direct-ICP work, or in Direct-HY-ICP applications.

Figure 2 indicates a typical HPLC-HY-ICP separation for a mixture of three arsenic oxyanion standards, all baseline resolved within 9 mins. The specific HPLC conditions used for this analysis are indicated in the Figure. Arsenite and arsenate are completely resolved from one another, and these have been the only two species commonly found in well water samples thus far (22). It is possible that other well water samples from different regions could contain organoarsenical species, in addition to or instead of these two.

The initial intent of utilizing continuous, on-line hydride generation with HPLC-ICP was to substantially lower the MDLs from those initially realized by HPLC-ICP alone (3). The current studies with these particular arsenic species have indicated a MDL improvement of at least one to two orders of magnitude by HPLC-HY-ICP, Table 1. The MDLs are defined here as the minimum concentration of analyte that produces a signal-to-noise ratio three times the standard deviation of the background noise level. This is a somewhat different definition of MDLs than that normally used by chromatographers, but it is actually the approach to MDLs generally employed by spectroscopists. We have discussed the potential advantages of using this approach to determining MDLs previously (3). It is clear from Table 2 that the ICP response for each arsenic species has been enhanced (improved) by at least a factor of about 1,000 over the similar response in the absence of any hydride generation. Theory would have predicted an MDL improvement, at best, of two orders of magnitude (100%), in going from HPLC-ICP to HPLC-HY-ICP. This is because in

Downloaded At: 17:09 24 January 2011

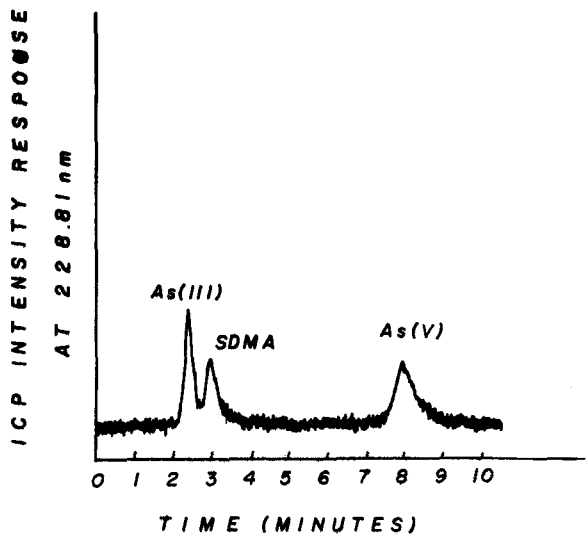


Figure 2. HPLC-HY-ICP chromatogram of arsenite (III), sodium dimethyl arsenate (SDMA), and arsenate (V) at the 200 ppb levels in each, using PIC A in the mobile phase at 1 ml/min flow rate with a 10 um, 25-cm C-18 reversed phase column and 200 ul injections.

Downloaded At: 17:09 24 January 2011

Table 1. HPLC-HY-ICP and HPLC-ICP Minimum Detection Limits (MDLs) (ppm)

COMPOUND NAME	HPLC-HY-ICP[a]	HPLC-ICP[b]
Arsenite	0.050	48.0
Arsenate	0.050	24.0
Sodium Dimethyl Arsenate (SDMA)	0.105	199.0

a. HPLC-HY-ICP conditions used an Excalibar RP-18 column, 4% $NaBH_4$ solution and concentrated HCL. Injection volume = 200 ul.
b. HPLC-ICP conditions used an Excalibar RP-18 column and injection volumes of 20 ul.

Downloaded At: 17:09 24 January 2011

Table 2. Analysis of Spiked Distilled Water Samples for Arsenate and Arsenite (ppb)

SAMPLE NO.	HPLC-HY-ICP[a]			HY-ICP	ACTUAL LEVELS			
	ARSENITE	ARSENATE	TOTAL	TOTAL	ARSENITE	ARSENATE	TOTAL	% ERROR[b]
1	52 ± 14[c]	39 ± 13[c]	91	93 ± 1	52	56	118	-23
2	74 ± 8	63 ± 6	137	119 ± 1	62	65	127	+8
3	54 ± 3	----	54	68 ± 0	84	--	84	-36
4	112 ± 50	173 ± 51	285	260 ± 2	87	141	228	+25
5	101 ± 33	69 ± 9	169	198 ± 4	104	97	201	-15
6	104 ± 45	88 ± 27	192	209 ± 4	104	97	201	-4
7	166 ± 5	---	166	124 ± 1	140	--	140	+19
8	271 ± 15	203 ± 13	474	407 ± 2	208	195	403	+17
9	322 ± 32	282 ± 32	604	509 ± 11	261	243	504	+19
10	301 ± 34	---	301	280 ± 1	279	--	279	+9
11	404 ± 122	428 ± 49	832	977 ± 9	397	527	924	-11

a. HPLC-HY-ICP conditions used an Excalibar RP-18 column, hydride conditions of 4% $NaBH_4$ solution and concentrated HCl.
b. % Error refers to difference between total arsenic content as determined by HPLC-HY-ICP and actual total arsenic levels spiked.
c. Numbers represent the average ± standard deviation for at least three separate runs made on the same day. Low standard deviations for HPLC-HY-ICP may be due to the small number of analyses done at each sample level.

the absence of hydride generation, only about 1% of the total HPLC eluent ever enters the ICP plume region, due to the current use of the cross flow nebulizer. With a 100% hydride formation occurring, presumably all of this should enter the plasma/plume region once it enters the cross flow nebulizer. This is due almost entirely to the greater volatility and gaseous nature of the arsine as opposed to arsenate or arsenite anions in solution. Our observation of a 1,000 fold improvement (lowering) of MDLs, Table 1, is due to the fact that we are here comparing a 20 ul injection by HPLC-ICP versus a 200 ul injection(s) by HPLC-HY-ICP. This therefore adds another order of magnitude to lowering of the final HPLC-HY-ICP MDL for almost all three species. Thus, MDLs with a mass sensitive detector such as the ICP as an HPLC-ICP system, could be routinely lowered just by going to larger injection volumes than the normally employed 20 ul or 25 ul.

The precision of the HPLC-HY-ICP method at the MDL was studied by injecting a standard mixture of arsenite and arsenate at the 50 ppb levels in each species. This solution was injected at least ten times (n=10), and each sample injection was preceded by a blank injection prepared as for the sample. The percent relative standard deviation (%RSD) was 16% for arsenite and 23% for arsenate. This is expected for a level determined near the MDL, where the theoretical precision would be 33% (signal-to-noise = 3:1).

Our observation in Table 1 that we are realizing at least a two fold lowering of the MDLs in going to HPLC-HY-ICP strongly suggests that we may have near 100% arsine conversion/formation efficiencies from arsenate and arsenite. However, we have not conclusively demonstrated this as yet. Complete conversion of the parent arsenic anions to their desired hydride form is not absolutely necessary, as long as the reaction extent occurs reproducibly.

Calibration plots for each of the three arsenic anions were linear over four orders of magnitude, ranging from the low part-per-billion (ppb) to mid-part-per-million (ppm) region. This includes the region of most interest for environmental samples. The correlation coefficients for these calibration plots ranged from 0.988 to 0.999.

Spiked Distilled Water Analysis

A series of eleven (11) spiked distilled water samples, at known concentration levels of both arsenite and arsenate, have been studied now by HPLC-HY-ICP, Table 2. These results indicate a general agreement between the levels of arsenic species spiked and the values determined. A linear regression analysis of these results and the actual spiked values gave correlation coefficients of 0.979 and 0.967 for arsenite and arsenate respectively. The total arsenic level in each of these samples were also determined by

Downloaded At: 17:09 24 January 2011

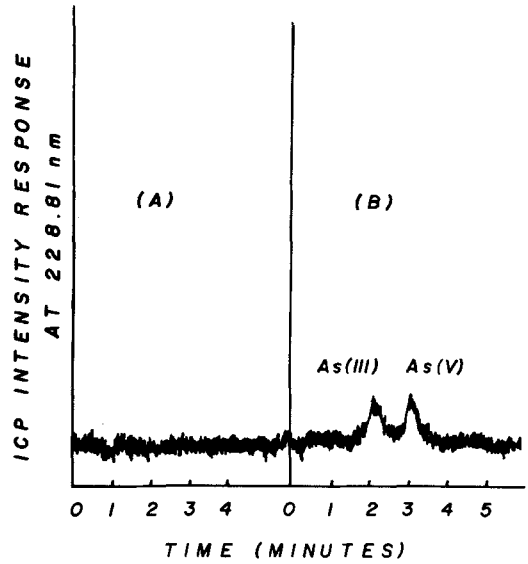


Figure 3. HPLC-HY-ICP chromatograms for (A) a 200 ul blank injection of distilled water; and (B) a 200 ul injection of a spiked distilled water sample prepared at the 40 ppb to 60 ppb levels in both arsenate and arsenite.

Downloaded At: 17:09 24 January 2011

Direct-HY-ICP, Table 2. In general, there is good agreement between these values and the actual total arsenic content. As one might expect, the % error tends to get worse at lower and lower concentration levels, especially as these approach the known, already demonstrated MDLs. Also, the accuracy of the individual arsenic determinations falls off as the spiked levels approach MDLs.

Figure 3 illustrates a typical HPLC-HY-ICP chromatogram of a spiked distilled water sample, with the concentration levels as indicated. Even at this level of 40 ppb to 60 ppb in each of the two arsenic anions, each HPLC peak is clearly discernible and substantially above the background noise levels. In going from a 25-cm long column to a 15-cm one, Figures 2 and 3, the total analysis time has now been cut about 50%.

Application to Environmental Well Water Sample Analysis

This HPLC-HY-ICP technique has now been applied to actual well water samples from the state of New Hampshire, wherein initial analyses for total arsenic content had demonstrated surprisingly high levels, at times 200 ppb to 250 ppb or thereabouts. Our methods of sample preservation have been indicated above (Experimental), but no chemical method was worked out by us

in order to effectively preserve arsenic species content. Other workers have demonstrated that ascorbic acid added to water samples can prevent the interconversion of arsenite to arsenate on standing (22). Our own interests have been in determining how much of species interconversion does occur in these particular well water samples on standing in a refrigerator, without the initial addition of any chemical preservatives. Table 3 indeed summarizes these results, wherein we have analyzed two such well samples as a function of time after their removal from the well itself. There is a relatively small decrease in the arsenite content and a corresponding increase in the arsenate levels when we compare Day 1 with Days 2 and 4. Such results suggest that analysis of these particular samples anywhere from Days 1 to 4 would probably provide accurate results for the levels of arsenic species originally present in the well itself. It is indeed possible that sample integrity, based on the relative changes in arsenite/arsenate from Days 1 to 4, could be retained for even one week after the samples were taken. However, by Day 19, which was the next point of analysis in this study, all of the initial arsenite had been converted to arsenate. Clearly, in order for this analysis to be absolutely representative of the arsenic species originally present in the well itself, samples should be analyzed immediately after collection in order to preserve sample integrity. Future utilization of HPLC-HY-ICP or alternative approaches for environmental water speciation should/must take sample preservation and sample integrity into consideration (22).

In order to determine species differences from one well to another, we have applied these techniques to another six well water samples, Table 4. As indicated in the last column on the right of Table 4, these samples were analyzed from six days to four months after they were collected, and stored as already indicated. Thus, for the first three samples, numbers 3-5, in all probability there has been complete conversion of any originally present arsenite to arsenate. This would clearly explain why these three samples only show the presence, at the time of their analysis, of arsenate. These results therefore do not necessarily represent what was originally present in the well water at the time of collection or in the well itself. However, the final values indicated for these particular samples are indicative of what was present at the time of analysis. On the other hand, for samples 6-8, these were analyzed within one week after the time of collection, and on the basis of our somewhat limited stability studies, Table 3, the speciation indicated here is probably indicative of what was originally present in these wells at the time of sample collection. The results by HPLC-HY-ICP and Direct-HY-ICP are consistent with regard to total arsenic present for a given sample on that day of analysis. Sample number 6 contains both arsenate and arsenite,

Downloaded At: 17:09 24 January 2011

Table 3. Determination of Arsenite and Arsenate Stability in Well Water Samples

SAMPLE NO.	As SPECIES[a]	DAY 1[b]	DAY 2[b]	Day 4[b]	Day 19[b]
1	ARSENITE	188 ± 18	126 ± 10	143 ± 5	0 ppb
	ARSENATE	56 ± 4	89 ± 23	76 ± 0	232 ± 40 ppb
	TOTAL	244 ± 22	216 ± 33	219 ± 5	232 ± 40 ppb
2	ARSENITE	183 ± 14	140 ± 13	156 ± 0	0 ppb
	ARSENATE	53 ± 14	75 ± 20	69 ± 4	216 ± 16 ppb
	TOTAL	236 ± 28	215 ± 33	225 ± 4	216 ± 16 ppb

a. HPLC-HY-ICP conditions used an Alltech HP Guard Column, 3% $NaBH_4$ and concentrated HCl for hydride generation.
b. All numbers represent the average ± standard deviation for at least three (3) separate analyses performed on the same working day.

Table 4. HPLC-HY-ICP and Direct-HY-ICP Analyses of Well Water Samples (ppb)

SAMPLE NO.	HPLC-HY-ICP[a, b]		TOTAL As	DIRECT-HY-ICP	DAYS OF
	ARSENATE	ARSENITE		TOTAL As	STORAGE
3	182 ± 4	ND[c]	182	212 ± 4	99
4	196 ± 12	ND	196	226 ± 1	86
5	235 ± 24	ND	235	243 ± 1	86
6	78 ± 14	99 ± 7	177	193 ± 1	7
7	ND	126 ± 3	126	153 ± 0	6
8	ND	ND	ND	7 ± 3	6

a. HPLC-HY-ICP conditions used an Excalibar or 15-cm in-house slurry packed RP-18 column, other conditions as indicated in text.
b. Numbers represent the average ± standard deviations (ppb) for at least three separate analyses performed on the same working day.
c. ND indicates that no arsenite or arsenate could be detected in the sample at or above the MDL of 50 ppb.

Table 5. External Standard Versus Standard Additions Method of Analysis

SAMPLE COMPONENT	EXTERNAL STANDARD METHOD	STANDARD ADDN. METHOD
ARSENITE	99 ± 7	103
ARSENATE	78 ± 14	81

Downloaded At: 17:09 24 January 2011

almost equally distributed, sample 7 contains only arsenite, clearly this has not converted yet to arsenate, and sample 8 contains no detectible arsenate or arsenite at these limits of detection.

The standard additions method was performed on two of the samples of Table 4, and Table 5 reports these results for sample number 6 above. Indicated here are the results of the direct analysis by the external standard method and the analogous results by the standard additions method. These overall results for the two different approaches on the same sample are identical within experimental error. A similar study with another well water sample of Table 4 provided results which also were identical within exptl. error. Of interest in Table 4 is the fact that samples 7 and 8 were drawn from different, but nearby wells, and these contain extremely different levels of arsenic species and total arsenic. This speciation approach could therefore have utility for tracing the path or source of underground arsenic contamination of drinking water supplies.

Downloaded At: 17:09 24 January 2011

CONCLUSIONS

We have developed and optimized new HPLC-HY-ICP approaches which can now provide a rapid and direct method of speciating well water supplies for total arsenic levels and individual arsenic species levels. Such approaches therefore provide an approach to determine variations of arsenic species between wells or other water supplies. The methods are totally usable down to the 50 ppb level for arsenate and arsenite, which is apparently at or below the demonstrated level of these arsenic species in those wells already studied here. It is hoped that these newer approaches will now find widespread acceptance and utilization by others interested in determining arsenic species levels in a variety of environmental, biological, industrial, and toxicological samples.

ACKNOWLEDGEMENTS

We wish to acknowledge the assistance of S. Colgan and R. Shansky at NU in their preparation of various spiked water samples. C. Baxter and C. Nelson of the N.H. Water Supply Division were helpful in providing us with various well water samples. This work was supported by a research and development contract from the Analytical Instrument Division of Instrumentation Laboratory, Inc. to NU, which has allowed us to undertake and complete the work described. Additional support was provided, in part, by a grant from the NIH Biomedical Sciences Research Support Grant No. RR07143, Department of Health and Human Services, to NU. We are very grateful for these sources of financial assistance. This is contribution number 153 from The Barnett Institute of Chemical Analysis and Materials Science at Northeastern Univ.

REFERENCES

1. Krull, I.S., in Liquid Chromatography in Environmental Analysis, ed. by Lawrence, J.F., The Humana Press, Clifton, N.J., in press, 1983, Chap. 5.

2. Krull, I.S. and Jordan, S., American Laboratory, 21 (October, 1980).

3. Bushee, D., Krull, I.S., Savage, R.N., and Smith, S.B., Jr., J. Liquid Chromatogr., 5(3), 463 (1982).

4. Florence, T.M., Talanta, 29, 345 (1982).

5. Krull, I.S., Panaro, K., and Gershman, L.L., J. Chrom. Sci., 21, 460 (1983).

6. Berman, E., Toxic Metals and Their Analysis, Heyden & Son, Ltd., London, 1980.

7. Doull, J., Klaasen, C.D., and Amdur, M.O., Toxicology-The Basic Science of Poisons, Second Edition, MacMillan Publ. Co., Inc., New York, 1980, Chapter 17.

8. Sigel, H., Metal Ions in Biological Systems. Carcinogenicity and Metal Ions, Volume 10, Marcel Dekker, New York, 1980.

9. Some Metals and Metallic Compounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 23. International Agency for Research on Cancer, Lyon, France, 1980.

10. Krull, I.S., Bushee, D., Savage, R.N., Schleicher, R.G., and Smith, S.B., Jr., Anal. Letters, 15(A3), 267 (1982).

11. Brinckman, F.E., Jewett, K.L., Iverson, W.P., Irgolic, K.J., Ehrhardt, K.C., and Stockton, R.A., J. Chromatogr., 191, 31 (1980).

12. Ricci, G.R., Shepard, L.S., Colovos, G., and Hester, N.E., Anal. Chem., 53, 610 (1981).

13. Grabinski, A.A., Anal. Chem., 53, 966 (1981).

14. Morita, M., Uehiro, T., and Fuwa, K., Anal. Chem., 53, 1806 (1981).

15. Fish, R.H., Brinckman, F.E., and Jewett, K.L., Environ. Sci. & Tech., 16, 174 (1982).

16. Irgolic, K.J., Stockton, R.A., Chakraborti, D., and Beyer, W., Spectrochim. Acta, 38B, 437 (1983).

17. Gast, C.H., Kraak, J.C., Poppe, H., and Maessen, F.J.M.J., J. Chromatogr., 185, 549 (1979).

18. Shaikh, A.U. and Tallman, D.E., Anal. Chim. Acta, 98, 251 (1978).

19. Nakahara, T., Anal. Chim. Acta, 131, 73 (1981).

20. Ebdon, L., Paper number 47 presented at the September, 1982 Federation of Analytical Chemistry and Spectroscopy Societies IXth Annual Meeting, Philadelphia, Penna.

21. Demko, P.R., Bushee, D., Krull, I.S., and Smith, S.B., Jr., Paper number 261 presented at the September, 1982 Federation of Analytical Chemistry and Spectroscopy Societies IXth Annual Meeting, Philadelphia, Penna.

Downloaded At: 17:09 24 January 2011

22. Lederer, W.H. and Fensterhein, R.J., Arsenic-Industrial, Biomedical, and Environmental Perspectives, van Nostrand Reinhold, Inc., New York, 1983, Chapter 22.

23. Ng, K.C. and Caruso, J.A., Anal. Chem., 55, 2032 (1983).

24. Nisamaneepong, W., Ng, K.C., and Caruso, J.A., Paper number 297 presented at the Federation of Analytical Chemistry and Spectroscopy Societies Xth Annual Meeting, Philadelphia, Penna., September, 1983.

25. de Oliveria, E., McLaren, J.W., and Berman, S.S., Anal. Chem., 55, 2047 (1983).

26. Abbreviations used: HPLC = high performance liquid chromatography; HY-ICP = hydride generation-inductively coupled plasma emission detection; HY = hydride generation; ICP = inductively coupled plasma emission detection; PHD = plasma hydride device; IL = Instrumentation Laboratory, Inc.,; ppb = parts-per-billion (ng/g, ng/ml); ppm = parts-per-million (ug/g, ug/ml); SDMA = sodium dimethylarsenate; cm = centimeter; mm = millimeter; MDLs = minimum detection limits; RP = reversed phase; GFAA = graphite furnace atomic absorption; DCP = direct current plasma; FAA = flame atomic absorption; UV = ultraviolet; LCEC = liquid chromatography-electrochemical detection; $NaBH_4$ = sodium borohydride; HCl = hydrochloric acid; NaOH = sodium hydroxide; HP = high performance; %RSD = percent relative standard deviation;

Downloaded At: 17:09 24 January 2011