Journal of Chromatography, 391 (1987) 161–168
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 258

HYDRIDE GENERATION–FLAME ATOMIC-ABSORPTION SPECTROMETRY AS AN ARSENIC DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

TAMIO MAITANI*, SADAO UCHIYAMA and YUKIO SAITO
National Institute of Hygienic Sciences, Kamiyoga 1-18-1, Setagaya, Tokyo 158 (Japan)
(First received July 25th, 1986; revised manuscript received November 10th, 1986)

SUMMARY

Hydride generation–flame atomic-absorption spectrometry (HG–FAAS) was used as a continuous detection system for arsenic in the eluate from high-performance liquid chromatography (HPLC). Four arsenic species (arsenite, arsenate, monomethylarsonate and dimethylarsinate) were detected separately with the HPLC–HG–FAAS system equipped with an anion-exchange column. When hijiki (*Hizikia fusiforme*) extract was examined, arsenate was found predominantly and arsenite and dimethylarsinate were also detected. Liver supernatant fraction obtained from mice administered orally with arsenite was also studied with the HPLC–HG–FAAS system equipped with a gel permeation column. In addition to free or low-molecular-weight ligand-bound arsenic, high-molecular-weight protein-bound arsenic fractions were also detected.

INTRODUCTION

The toxic actions of arsenic species are highly dependent on their valence states and chemical forms[1]. Further, inorganic arsenic ingested by man and animals is methylated *in vivo* to give organoarsenic compounds such as monomethylarsonate (MMA), dimethylarsinate (DMA) and trimethylarsenic compounds[2–4]. Therefore, it is essential to separate and determine the individual arsenic species in order to evaluate the toxicity of arsenic. Various separative methods have been reported[5–8], including high-performance liquid chromatography (HPLC)[9]. The distribution of ingested arsenic in tissue cytosol fraction may be important information in toxicological studies, because the tissue retention of arsenic bound to high-molecular-weight proteins (HMWP) is longer than those in other fractions[10]. In this field, HPLC equipped with a gel permeation column may be a useful technique[11,12].

To monitor metal levels in the eluate from an HPLC column, direct connection of the HPLC system with a spectrometer by means of which metal levels can be

* Parts of this study were presented at the *50th Symposium of the Food Hygiene Society of Japan, Niigata, October 17–18th, 1985.*

0021-9673/87/$03.50

continuously monitored is desirable. Suzuki[13] devised a direct connection between HPLC and flame atomic-absorption spectrometric (FAAS) instruments for metallo-protein analysis. However, FAAS has a poor sensitivity towards arsenic. Although the graphite furnace atomic-absorption spectrometer has a higher sensitivity and has been used as a detector for arsenic[14–16] or selenium[17] in HPLC, continuous monitoring is not possible. Inductively coupled plasma–atomic emission spectrometry (ICP–AES) has also been used for the detection of arsenic in HPLC with[18] and without[19–21] hydride generation (HG). Although ICP–AES has a high sensitivity to arsenic, the spectrometer is expensive.

HG–FAAS has been widely used for the determination of several elements including arsenic because of its high sensitivity. This system is relatively inexpensive and capable of continuous monitoring. In this work, we studied the direct connection of HPLC with HG–FAAS and this system was applied to food and tissue cytosol samples.

EXPERIMENTAL

Reagents

Sodium *m*-arsenite and sodium arsenate were obtained from Sigma (St. Louis, MO, U.S.A.), sodium monomethylarsonate from Carlo Erba (Milan, Italy) and sodium dimethylarsinate and sodium tetrahydroborate ($NaBH_4$) from Wako (Osaka, Japan). All other chemicals were of analytical-reagent grade.

Standards

Stock solutions (1000 ppm) of the arsenic compounds were prepared in distilled water. They were used within 5 days. Standard solutions were freshly prepared by serial dilutions of the stock solutions before use.

Preparation of samples

A 1-g portion of a commercially available hijiki (*Hizikia fusiforme*) sample was soaked in 30 ml of distilled water for 20 h and filtered through a Toyo filter-paper 5A (Toyo Roshi Co. Ltd., Tokyo, Japan). After the total volume had been measured, a portion of the filtrate was subjected to HPLC. Male mice of ICR strain (Charles River Japan Co., Atsugi, Japan) were administered orally with sodium arsenite at a dose of 40 mg/kg body weight of As and killed 1 h later. Livers were excised, washed in cold saline solution and homogenized in 3 volumes of 0.1 *M* Tris–HCl buffer solution (pH 7.4, 0.25 *M* glucose). The homogenate was centrifuged at 100 000 *g* for 60 min and the supernatant fraction was subjected to HPLC.

HPLC–HG–FAAS

A mixture of standard solutions or hijiki extract was subjected to HPLC on a Varian Model 5000 system equipped with an anion-exchange column (Nucleosil 10SB; Macherey, Nagel & Co., Düren, F.R.G.) (250 × 4.6 mm I.D.) and the column was eluted with 50 m*M* phosphate buffer (pH 6.75) at a flow-rate of 0.5 ml/min. A 100-μl portion of liver supernatant fraction was also applied to the HPLC system equipped with a gel permeation column (TSK GEL G3000SW; Toyo Soda) (600 × 7.5 mm I.D.) with a TSK SW guard column (75 × 7.5 mm I.D.) and eluted with

50 m*M* Tris–HCl buffer solution (pH 7.2) at a flow-rate of 1 ml/min. The eluate from each column was introduced into a hydride generation apparatus (Varian VGA-76) and, unless indicated otherwise was mixed successively with 10% oxalic acid or concentrated hydrochloric acid (1 ml/min) and then with 3% $NaBH_4$ solution (1 ml/min) supplied continuously by peristaltic pumps. The gaseous arsine derivatives produced in a reaction coil (600 mm × 0.5 mm I.D.) were separated from the liquid phase through a gas–liquid separator and introduced by nitrogen into a quartz absorption cell of an FAAS instrument (Varian AA-775 with R106UH photomultiplier tube) heated with an air–acetylene flame. The absorption of arsenic was continuously monitored by FAAS.

RESULTS AND DISCUSSION

The utilization of $NaBH_4$ solution instead of zinc (solid) as a reducing agent made the continuous reduction of arsenic compounds to volatile hydrides possible. Therefore, the HG–FAAS method can be used as a continuous detection system for arsenic compounds in HPLC eluates. A schematic diagram of the HPLC–HG–FAAS system assembled in this study is depicted in Fig. 1. In the HPLC–FAAS system (without an HG apparatus), the discrepancy between the flow-rate in HPLC and the uptake rate in FAAS is a significant problem, because the outlet of the HPLC column is directly connected with the FAAS nebulizer tube. In HPLC–HG–FAAS, on the other hand, there were no such problems, as the volatile hydrides produced were separated from the eluate and the gaseous hydrides were carried by an inert gas (nitrogen in this study) to a quartz absorption cell attached to the FAAS instrument.

Among the commonly found arsenic species, arsenite (inorganic trivalent state) is the most toxic[1]. The toxic arsenite is methylated to the much less toxic organoarsenic compounds[2–4]. Therefore, it is advantageous to detect arsenite selectively in various mixtures of arsenic compounds in the HPLC eluate, especially equipped with a gel permeation column. In HG–FAAS, the observed peak heights of arsenic species are dependent on the types of acids or buffers mixed with $NaBH_4$ solution. This means that the selective detection of arsenite may be possible by selecting suitable acids or buffers. The acid dependence of the peak height for arsenite, arsenate, MMA,

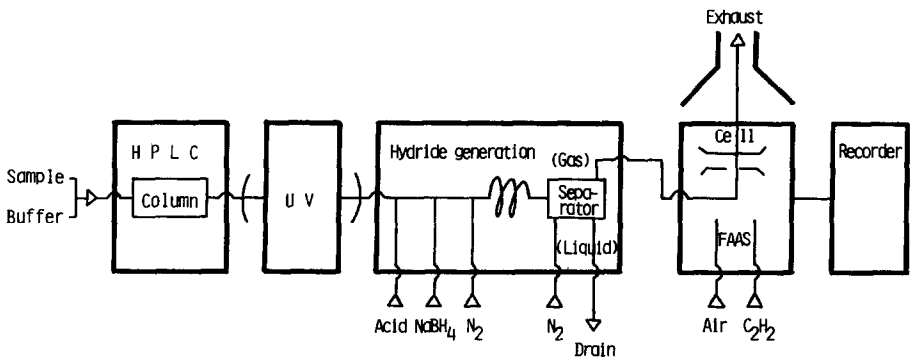

Fig. 1. Schematic diagram of HPLC–HG–FAAS system. HPLC = Model 5000 high-performance liquid chromatograph (Varian); hydride generation = VGA-76 hydride generation apparatus (Varian); FAAS = AA-775 flame atomic-absorption spectrometer (Varian).

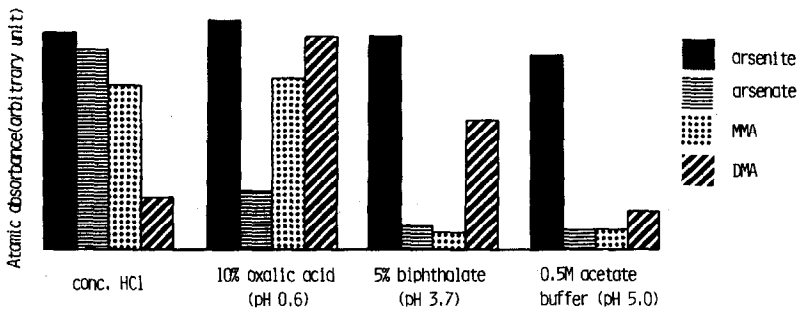

Fig. 2. Dependence of atomic absorbance of arsenic species on the acids used in hydride generation. Each arsenic concentration is 10 ppb; sample flow-rate, 1 ml/min; acid or buffer, 1 ml/min; 3% $NaBH_4$, 1 ml/min. MMA = Monomethylarsonate; DMA = dimethylarsinate.

and DMA observed by using several acids or buffers and 3% $NaBH_4$ solution is shown in Fig. 2. $NaBH_4$ was dissolved in distilled water instead of sodium hydroxide solution to examine the effects of weak acids, buffers and concentrated hydrochloric acid. Arsenite showed an almost constant absorption regardless of the acids or buffer and the other three species showed marked acid dependence. As arsenic species other than arsenite showed some atomic absorption under the four conditions used (Fig. 2), conditions for the completely selective detection of arsenite could not be found.

Fig. 3 shows the effect of $NaBH_4$ concentration on the peak heights observed for the four arsenic species. As MMA and DMA showed the largest peaks at a concentration of 3%, the concentration of $NaBH_4$ was fixed at 3% in the experiment below (it was not practical to prepare a more concentrated solution of $NaBH_4$ because of its bubbling). In this study, $NaBH_4$ was dissolved in distilled water and it was not intended to make the observed peak heights for the four arsenic species equal. However, if hydrochloric acid only is used as an acid, the use of a higher concentration of $NaBH_4$ dissolved in sodium hydroxide solution may result in equal peak heights for the four arsenic species[22].

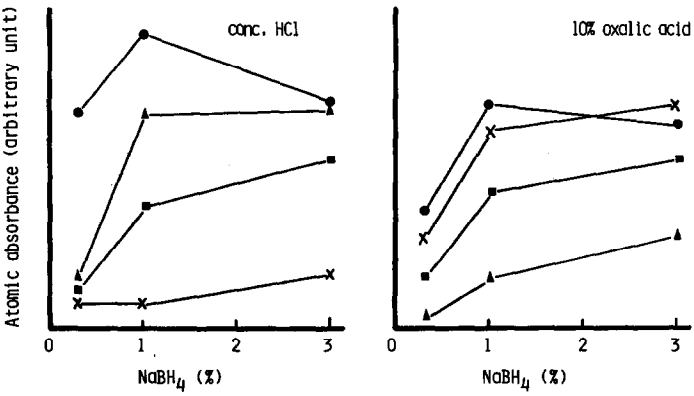

Fig. 3. Dependence of arsenic species on the concentration of $NaBH_4$. ● = Arsenite; ▲ = arsenate; ■ = monomethylarsonate; × = dimethylarsinate. Sample flow-rate, 1 ml/min; acid, 1 ml/min; $NaBH_4$, 1 ml/min.

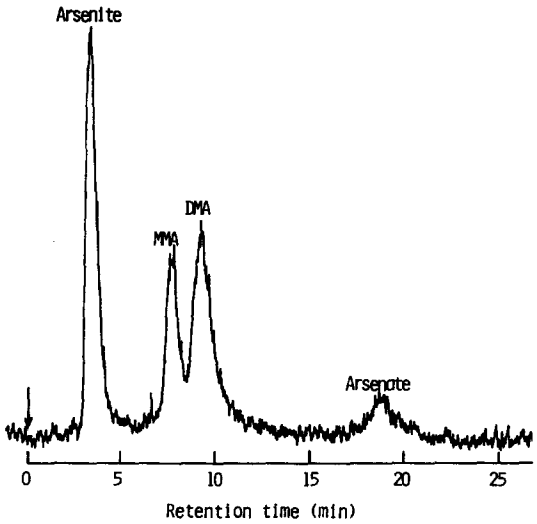

Fig. 4. Separation and detection of four arsenic species by HPLC–HG–FAAS with an anion-exchange column (Nucleosil 10SB). A 10-ng amount of each species was applied; sample flow-rate, 0.5 ml/min; 10% oxalic acid, 1 ml/min; 3% $NaBH_4$, 1 ml/min. MMA = Monomethylarsonate; DMA = dimethylarsinate.

Ion-exchange column

A mixture of the four arsenic species was applied to an anion-exchange column (Nucleosil 10 SB[19]) and the eluate was monitored continuously by HG–FAAS (Fig. 4). As an acid, 10% oxalic acid was used for the chromatogram to show the presence of DMA in hijiki extract (see below). The assignments of the peaks were made by applying standard solutions to the system. The four species were detected separately (the detection limits under the conditions used were 1, 7, 2 and 2 ng of arsenic for arsenite, arsenate, MMA and DMA, respectively, at a signal-to-noise ratio of 2). The overall detection limits were of the same level as those in HPLC–ICP–AES (2.6 ng)[19].

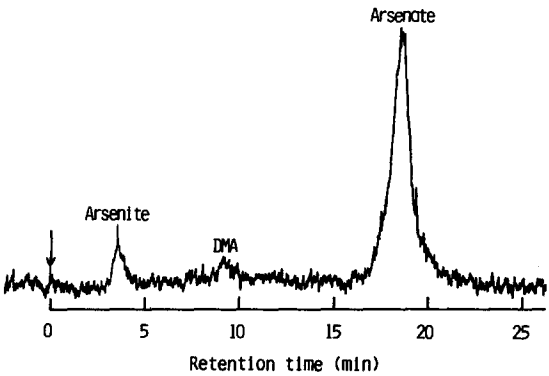

Fig. 5. Chromatogram of hijiki extract obtained by HPLC–HG–FAAS with anion-exchange column. A 50-μl portion of the extract was injected; sample flow-rate, 0.5 ml/min; 10% oxalic acid, 1 ml/min; 3% $NaBH_4$, 1 ml/min. DMA = Dimethylarsinate.

It is well known that a large amount of arsenic is present in many seaweeds[23]. Thus, hijiki (a seaweed) extract (2.1 ppm total As, 50 μl) was subjected to HPLC–HG–FAAS using an anion-exchange column. The chromatogram is presented in Fig. 5. The main peak and the small peaks were ascribed to arsenate and arsenite and DMA, respectively, based on the retention times. Some workers have also reported the dominant presence of arsenate in hijiki extract based on other methods[19,24–26].

Gel permeation column

Liver supernatant fraction obtained from mice administered orally with arsenite was subjected to HPLC–HG–FAAS using a gel permeation column (Fig. 6). The peak at 28 min was ascribed to free or low-molecular-weight ligand (LMWL)-bound arsenite, because arsenite spiked in a liver supernatant fraction from control mice also gave its arsenic peak at the expected retention time. As ingested inorganic arsenic is methylated *in vivo*[27,28] and arsenate, MMA and DMA added to the control liver supernatant fraction were not fully resolved on the gel permeation column, the sharp peak at 24 min was tentatively assigned as a mixture of the three free (or LMWL-bound) arsenic species. As the peak height was reduced to one-third when the eluate was monitored by using 10% oxalic acid (the other peaks retained their heights) (data not shown), arsenate seems to contribute mainly to the peak (*cf.*, Fig. 2). It is not known whether the administered arsenite was oxidized to arsenate *in vivo* or in the experimental procedures.

The peaks at shorter retention times seem to be due to HMWP-bound arsenic, as it has been reported that a significant portion of arsenic in liver cytosol is bound to HMWPs[10]. The HMWPs might be identical with those proteins to which cadmium was bound in mouse tissue supernatant fractions after injection of cadmium, as observed in our previous studies[12,29], because both arsenite[30] and cadmium strongly

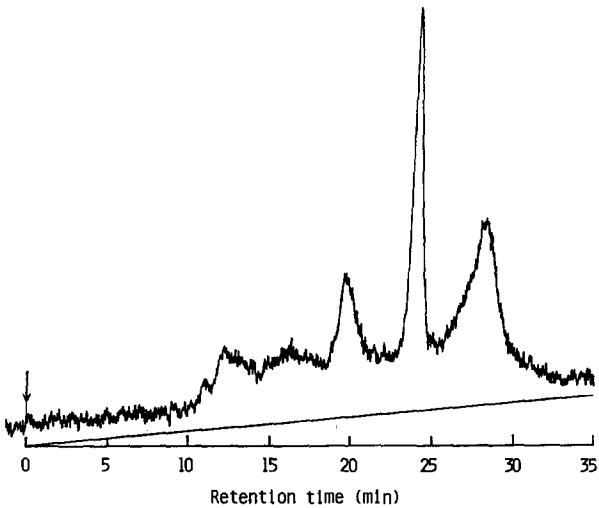

Fig. 6. Chromatogram of liver supernatant fraction after a single oral administration of arsenite to mice obtained by HPLC–HG–FAAS using the TSK G3000SW gel permeation column. A 100-μl portion of the supernatant fraction was injected; sample flow-rate, 1 ml/min; concentrated hydrochloric acid, 1 ml/min; 3% $NaBH_4$, 1 ml/min.

bind with sulfhydryl groups of proteins. Hence this system seems to be applicable to HMWP-bound arsenic which is dissociated by acids, and may serve to investigate the distribution of arsenic in the tissue cytosol fraction after arsenic administration, if consideration given to the non-selective adsorption and desorption of the metal to and from the packed materials. Although the arsenic species are not identified when using a gel permeation column, fractionation of the eluate and subsequent application of an ion-exchange column may clarify the arsenic species in the respective fractions.

Recently, Tye *et al.*[31] reported the connection of HPLC (with ion-exchange columns) with HG–FAAS and applied their system to water samples. In that study, two types of anion-exchange columns were used for the separation of the four arsenic species (arsenite, arsenate, MMA and DMA) and the eluents were switched for the separation. We studied more practical samples (food and tissue cytosol) to show that HG is not interfered by the coexisting constituents, and used one column and one eluent for each sample to demonstrate that HG–FAAS can be used generally as a continuous detection system for arsenic in the eluate from HPLC columns. Therefore, in addition to an ion-exchange column, a gel permeation column was also used. With the ion-exchange column (Nucleosil 10SB), four arsenic species were detected separately and the main arsenic species in hijiki extract was identified as arsenate. With the gel permeation column (TSK GEL G3000SW), HMWP-bound arsenic was detected in the liver supernatant fractions after arsenite administration.

In conclusion, HG–FAAS has been used successfully as a sensitive continuous detection system for arsenic in the eluate from HPLC columns. However, it is natural that this system is not applicable to arsenic species that cannot be converted into volatile hydrides.

REFERENCES

1 Committee on Medical and Biologic Effects of Environmental Pollutants, National Research Council, *Arsenic, Medical and Biologic Effects of Environmental Pollutants*, National Academy of Sciences, Washington, DC, 1977, Ch. 5.
2 G. K. H. Tam, S. M. Charbonnean, F. Bryce, C. Pomroy and E. Sandi, *Toxicol. Appl. Pharmacol.*, 50 (1979) 319–322.
3 J. P. Buchet, R. Lauwerys and H. Roels, *Int. Arch. Occup. Environ. Health*, 48 (1981) 111–118.
4 H. Yamauchi and Y. Yamamura, *Toxicology*, 34 (1985) 113–121.
5 L. R. Overby, S. F. Bocchieri and R. L. Fredrickson, *J. Assoc. Off. Anal. Chem.*, 48 (1965) 17–22.
6 Y. Talmi and D. T. Bostick, *Anal. Chem.*, 47 (1975) 21435–2150.
7 R. S. Braman, D. L. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker, *Anal. Chem.*, 49 (1977) 621–625.
8 H. Munz and W. Lorenzen, *Fresenius' Z. Anal. Chem.*, 319 (1984) 395–398.
9 R. Iadevaia, N. Aharonson and E. A. Woolson, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 742–746.
10 F. Bertolero, E. Marafante, J. E. Rade, R. Pietra and E. Sabbioni, *Toxicology*, 20 (1981) 35–44.
11 K. T. Suzuki, T. Motomura, Y. Tsuchiya and M. Yamamura, *Anal. Biochem.*, 107 (1980) 75–85.
12 T. Maitani and K. T. Suzuki, *Toxicol. Appl. Pharmacol.*, 62 (1982) 219–227.
13 K. T. Suzuki, *Anal. Biochem.*, 102 (1980) 31–34.
14 R. A. Stockton and K. J. Irgolic, *Int. J. Environ. Anal. Chem.*, 6 (1979) 313–319.
15 R. Iadevaia, N. Aharonson and E. A. Woolson, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 742–746.
16 J. F. Lawrence, P. Michalik, G. Tam and H. B. S. Conacher, *J. Agric. Food Chem.*, 34 (1986) 315–319.
17 D. R. Roden and D. E. Tallman, *Anal. Chem.*, 54 (1982) 307–309.
18 D. S. Bushee, I. S. Krull, P. R. Demko and S. B. Smith, Jr., *J. Liq. Chromatogr.*, 7 (1984) 861–876.

19 M. Morita, T. Uehiro and K. Fuwa, *Anal. Chem.*, 53 (1981) 1806–1808.
20 K. E. Lawrence, G. W. Rice and V. A. Fassel, *Anal. Chem.*, 56 (1984) 289–292.
21 W. D. Spall, J. G. Lynn, J. L. Andersen, J. G. Valdez and L. R. Gurley, *Anal. Chem.*, 58 (1986) 1340–1344.
22 M. Vahter, L. Friberg, B. Rahnster, A. Nygren and P. Nolinder, *Int. Arch. Occup. Environ. Health*, 57 (1986) 79–91.
23 G. Lunde, *Environ. Health Perspect.*, 19 (1977) 47–52.
24 H. Yamauchi and Y. Yamamura, *Jpn. J. Public Health*, 27 (1980) 647–653.
25 S. Fukui, T. Hirayama, M. Nohara and Y. Sakagami, *J. Food Hyg. Soc.*, 22 (1981) 513–519.
26 A. Yasui, C. Tsutsumi and S. Toda, *Agric. Biol. Chem.*, 47 (1983) 1349–1351.
27 H. Yamauchi, M. Iwata and Y. Yamamura, *Jpn. J. Ind. Health*, 22 (1980) 111–121.
28 J. P. Buchet, R. Lauwerys and H. Roels, *Int. Arch. Occup. Environ. Health*, 48 (1981) 71–79.
29 T. Maitani, Y. Saito and K. T. Suzuki, *Toxicology*, 37 (1985) 27–38.
30 A. E. Martell, *Environ. Health Perspect.*, 40 (1981) 207–226.
31 C. T. Tye, S. J. Haswell, P. O'Neill and K. C. C. Bancroft, *Anal. Chim. Acta*, 169 (1985) 195–200.