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GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETERS AS AUTOMATED ELEMENT-SPECIFIC DETECTORS FOR HIGH-PRESSURE LIQUID CHROMATOGRAPHY

THE DETERMINATION OF ARSENITE, ARSENATE, METHYLARSONIC ACID AND DIMETHYLARSINIC ACID

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SUMMARY

Techniques for the determination of trace element compounds at ppb** and ppm levels (in contrast to the determination of the total element concentration) are a prerequisite for the study of the transformations of trace elements in biological systems and the interactions of trace element compounds with biologically important molecules. Two automated high-pressure liquid chromatography (HPLC) systems with element-specific detectors, capable of detecting, identifying and quantitating trace element compounds were developed independently in our laboratories. One of the detectors consists of a Perkin-Elmer graphite furnace atomic absorption spectrometer (GFAA) and a specially adapted autosampler, whereas a Hitachi-Zeeman GFAA, a sample valve, an injector and associated electronics to control the analysis sequence comprise the components of the other detector. The capability of these systems to speciate trace element compounds is demonstrated using arsenite, arsenate, methylarsonic acid (MAA) and dimethylarsinic acid (DMAA) as examples. Arsenite, MAA and DMAA were successfully separated and quantitatively determined on a strong anion-exchange column with an aqueous acetate buffer as mobile phase. Arsenite, DMAA and arsenate can be speciated with a strong cation-exchange column using an aqueous solution of ammonium acetate as mobile phase. All four arsenic compounds can be separated on a C_{18} reversed-phase column with methanol–water mixtures saturated with tetraheptylammonium nitrate. These compounds can be identified and quantified in solutions containing 10–20 ppb arsenic. Sample sizes required for GFAA analysis vary between 20 and 100 μl. The separation schemes developed for the four arsenic compounds were used to speciate these compounds in

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** Throughout this article, the American billion (10^9) is meant.

soil extracts and drinking waters. The separation efficiency achieved thus far can very likely be improved through development of better column materials and mobile phases. This work with arsenic compounds clearly shows the great potential of these HPLC–GFAA analytical systems in the area of environmental trace element chemistry, in the field of physiological chemistry and in trace element-related nutritional studies.

INTRODUCTION

It is now firmly established that many trace element compounds experience extensive chemical transformations in the environment which may be caused by purely "chemical" means without participation of living organisms or may be biologically mediated[1]. Arsenic[2], selenium[3], mercury[4] and tin[5] can be cited as examples of trace elements which are subject to extensive transformations under appropriate conditions. The number of trace element compounds, which can be formed in the environment, is much larger than the number of compounds added to the environment by human activity or primary natural processes such as weathering of rocks. Each one of these trace element compounds possesses unique physical properties and chemical characteristics and causes specific beneficial or detrimental effects in its interaction with living systems. To understand the role of a trace element in the environment now means to understand the role of each particular trace element compound. The determination of the "total trace element concentration" in a sample does not provide any information on the presence or absence and on the concentrations of individual trace element compounds. The techniques for the determination of trace element compounds in environmental samples (in contrast to the determination of total element concentration) must be developed and refined before the transformations of trace elements in biological systems and the interactions of trace element compounds with biologically important molecules can be studied on a molecular level.

Many trace element compounds are not volatile enough to allow their gas chromatographic detection. High-pressure liquid chromatography (HPLC) techniques are ideal for the determination of such compounds. Samples prepared from biological materials contain trace element compounds at ppb to ppm levels in a matrix consisting of many other organic and inorganic compounds. Because investigators would like to find out primarily how many compounds of a specific trace element are in a sample and to determine their concentrations and chemical nature, the common, non-element specific HPLC detectors (refractive index, ultraviolet) are of very limited utility. A graphite furnace atomic absorption spectrometer (GFAA) combines the advantage of element specificity with high sensitivity for many elements. Two automated systems combining HPLC with GFAA as an element-specific detector were independently developed. The first system uses a Perkin-Elmer GFAA with a specially adapted autosampler[6]. The second system employes a Hitachi-Zeeman GFAA with a sample valve, an injector and associated electronics to control the analysis sequence[7].

This paper describes the separation and determination of arsenite, arsenate, methylarsonic acid (MAA) and dimethylarsinic acid (DMAA) using the HPLC–GFAA systems with strong cation (SCX) and anion-exchange (SAX) resins as well as reversed-phase columns. These analytical procedures were then applied to the determi-

nation of the arsenic compounds in soil extracts and drinking water as an example of the versatility of the HPLC–GFAA method.

EXPERIMENTAL*

Chemicals

Sodium arsenite ($NaAsO_2$), MAA, disodium methylarsonate (DSMA), DMAA, sodium dimethylarsinate (SDMA) used in the experiments with the Perkin-Elmer atomic absorption (AA) detector and three United States Department of Agriculture (USDA) soil samples which had been previously treated with arsenite, DSMA or monosodium methylarsonate (MSMA) were obtained from Dr. E. Woolson, USDA Agricultural Research Service, Beltsville, Md, U.S.A. All other chemicals were purchased from commercial sources and were used as received. The concentrations of solutions, prepared with deionized water, were based on the individual chemical assays provided by the manufacturers.

Separation of arsenic compounds on ion-exchange columns

Instrumentation. Altex Scientific, Model 110A single piston and Model 100 dual piston liquid chromatographic pumps were used to deliver solvents and analytes through SAX columns (25 cm × 3.2 mm I.D.) packed with 10-μm particles. The columns were purchased from Altex Scientific. The HPLC–GFAA system consisted of an Altex single-wavelength detector (254 nm) arranged in series to a Perkin-Elmer Model 360 atomic absorption spectrometer equipped with a programmable graphite furnace and a Perkin-Elmer autosampler Model AS-1 which periodically transferred 20-μl aliquots from a 50-μl flow-through sample cup to the graphite furnace for analysis. A detailed description of the system, its mode of operation and its detection capabilities were published[6].

HPLC–GFAA operational parameters. Before use approximately 60 ml of the mobile phase to be employed were passed through the column. Depending on the desired separation, the solutions were chromatographed on SAX or SCX columns at flow-rates ranging from 0.15 ml/min to 1.0 ml/min. Aqueous buffer solutions in the pH range 2.9 to 6.9 were employed with total concentrations of the anionic components of the buffers between 0.025 *M* and 0.075 *M*. Volumes of 20 μl of solutions containing the desired concentrations of one or any combination of the arsenic compounds to be investigated were injected into the columns. For the GFAA analyses 20-μl samples of the column effluent were injected automatically into the graphite cuvettes every 45 sec. The samples were dried in the graphite cuvette for 15 sec at 150° and for 5 sec at 200° and then atomized at 2700° for 10 sec. Arsenic concentrations were measured at 193.7 nm. The samples were not subjected to an ashing cycle to minimize volatilization. During atomization the "interrupt" mode of operation was employed, assuring a longer residence time of the arsenic atoms in the AA optical path. For automatic background correction a D_2-lamp was used.

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* Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental conditions and procedures. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material and equipment identified is necessarily the best available for the purpose.

Extraction of arsenic compounds from soils. A 25-ml volume of 1.0 *M* NH_4Cl was added to a 125-ml erlenmeyer flask containing 0.5 g of a soil treated with arsenic compounds. The flask was shaken on a Burrel wrist-action shaker for 30 min. After the mixture had settled for 5 min, the supernatant was decanted into 15-ml culture tubes and centrifuged for 20 min at 1470 *g*. In turn, two 1.5-ml samples, one from each supernatant were ultracentrifuged (15,100 *g*, 5 min), then analyzed for total arsenic by AA and arsenic compounds by HPLC–GFAA.

The residual soil from the above extraction was shaken for 17 h with 25 ml of solution 0.5 *M* and 0.1 *M* with respect to NH_4F and NaOH, respectively. The supernatant was treated as described above.

Separation of arsenic compounds on C_{18} reversed-phase columns

Instrumentation. A Waters Assoc. Model 6000 A dual piston pump was used to force the mobile phase through an Altex C_{18} reversed-phase column (25 cm × 3.2 mm I.D.) packed with 10-μm particles. The analytes were placed on the column through a Waters Assoc. Model UK6 sample injector. The detector system consisted of a Beckman Model 25 variable-wavelength UV–VIS spectrophotometer in series with a Waters Assoc. differential refractometer Model 401 and an Altex slider injection eight-port valve. This pneumatically activated sampling valve delivered 40 μl of the column effluent through an injector into the graphite cuvette of a Hitachi-Zeeman GFAA Model 170-70. An outline of the interface between the HPLC and the GFAA is available in the literature[7].

HPLC–GFAA operational parameters. Before analysis the columns were conditioned by pumping the mobile phase through the columns at 1 ml/min for 1 h. Solutions containing 20 ppm of arsenic of each of the compounds to be investigated were employed for the separation and calibration experiments. The amounts of arsenic compounds were varied by injecting volumes in the range 5 μl to 25 μl onto the column. Water–methanol mixtures 0.005 *M* with respect to tetrabutylammonium hydroxide, the pH of which was adjusted with phosphoric acid or water–methanol mixtures saturated with tetraheptylammonium nitrate ($\approx$ 0.002*M*), for which the pH was adjusted with 0.2 *M* sodium hydroxide, served as mobile phases. Flow-rates of 0.5 and 1.0 ml/min were used. For the GFAA analyses 40-μl samples of the column effluent were automatically injected into the graphite cuvette every 30 to 45 sec. The samples were dried in the graphite cuvette at 75° for 10 sec, ashed at 300° for 10 sec and atomized at 2500° for 7 sec. The carrier and sheath gas-flows were adjusted to 0.2 and 3.2 l/min, respectively. The carrier gas flow was interrupted during the atomization of the sample to increase sensitivity. Arsenic was determined using the 193.7 nm line.

Analysis of arsenic compounds in tap water samples. Amounts of arsenite, arsenate, MAA and DMAA required to obtain solutions containing 5.00 ppm arsenic of each arsenic compound was added to a sample of tap water from the local drinking water supply system. The solutions were filtered through a 0.45 μm filter. Samples (100 μl) were placed onto the column and analyzed by the HPLC–GFAA technique as described above.

RESULTS AND DISCUSSION

In this paper the names arsenite, arsenate, MAA, methylarsonate, DMAA and

dimethylarsinate, and the formulas $NaAsO_2$, Na_2HAsO_4, $CH_3AsO_3H_2$, $CH_3AsO_3Na_2$, $(CH_3)_2AsO_2H$ and $(CH_3)_2AsO_2Na$ used in the text, in tables and in figures do not imply that all of the arsenic compounds are present in the analyte, on the column, in the effluent or in the graphite cuvette as the particular forms (dissociated or undissociated) designated by the names and/or formulas. The degree of dissociation and the counter-ion is determined by the composition of the mobile phase. The names and formulas provide the composition of the arsenic compounds used in the study as given on the reagent bottles.

Previous techniques for the speciation of inorganic and methylated arsenic compounds relied on their reduction by $NaBH_4$ to volatile arsines, thereby causing extensive perturbations in the matrices containing these species and in the loss of information concerning the chemical nature of the compounds present in the sample[8]. The volatile arsines were subsequently analyzed employing microwave emission spectrometers. HPLC using SAX and SCX columns or C_{18} reversed-phase columns are capable of separating inorganic and organic arsenic compounds with no chemical pretreatment of the samples. At a flow-rate of 1 ml/min less than 10% of the sample is used for and destroyed during the analysis.

Separations on SAX columns

SAX column successfully separated dimethylarsinate, methylarsonate and arsanilic acid employing aqueous 0.025 *M*–0.050 *M* NaH_2PO_4 solutions of pH 4.4–4.5 as the mobile phase. (Fig. 1). The phosphate buffers of lower concentration produced longer retention times and larger AA signals than the more concentrated phosphate solutions. It has been reported that an excess of phosphate reduces the sensitivity of GFAA for the determination of arsenic[9]. Therefore, acetate buffers were investigated and found to be superior to the phosphate buffers. Several experiments were carried out to devise a procedure for the simultaneous separation of arsenate, arsenite, methylarsonate and dimethylarsinate using buffer solutions with several ammonium acetate–acetic acid ratios. Arsenate was too strongly retained when these buffer solutions, 0.075 *M* acetic acid (pH 3.00) or 0.075 *M* ammonium acetate (pH 6.93) were employed as eluents. Arsenite, methylarsonate and dimethylarsinate also had unacceptably long retention times with 0.075 *M* acetic acid as the mobile phase. Under neutral conditions (0.075 *M* ammonium acetate) these three compounds were eluted together with only slight retention. After evaluating solutions of intermediate pH, elution systems of approximately 0.04 *M* concentration with respect to acetic acid and ammonium acetate were found to separate arsenite, methylarsonate and dimethylarsinate (Fig. 2).

The GFAA sensitivity for the determination of an element is many times drastically influenced by matrix effects. To obtain information about these effects on the determinations of arsenic compounds, 0.05 *M* citric acid solutions, as an example of a buffer suitable for experiments to biologically transform arsenic compounds, were spiked with arsenite and dimethylarsinate and the resulting solutions analyzed by HPLC–GFAA with and without D_2 background correction. The results, summarized in Table I, show that background correction reduces the arsenic signal by 10 to 40% and indicate that 0.05 *M* citric acid solutions can produce signals almost comparable to solutions of arsenic compounds in distilled water.

The retention times and volumes of the arsenic compounds determined with

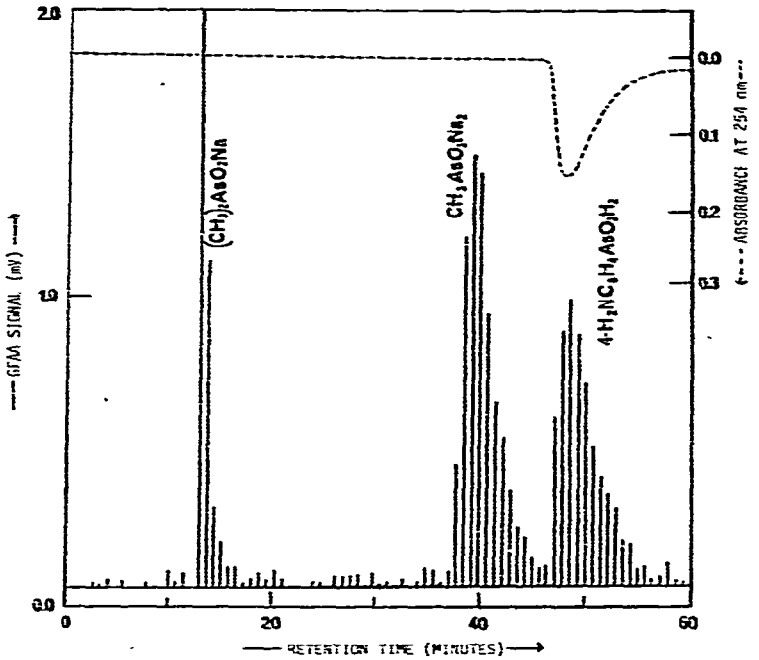

Fig. 1. Separation of SDMA, DSMA and arsanilic acid by the HPLC–GFAA technique (Perkin-Elmer GFAA) employing a SAX column: Altex LiChrosorb SAX column, 25 cm × 3.2 mm I.D., 10-μm particles, 20-μl sample containing 500 ng arsenic of each compound placed on column; mobile phase 0.05 *M* NaH_2PO_4; flow-rate 0.5 ml/min; 20 μl of column effluent injected into graphite cuvette every 45 sec; no background correction. Upper dashed curve shows the UV-detector signal which is sensitive only to the phenyl chromophore in arsanilic acid.

standard solutions employing distilled water as solvent may change when the arsenic compounds are present in more complicated solution matrices. Several chromatographic separations of arsenite, dimethylarsinate and methylarsonate were performed under conditions specified in Fig. 2 with the exception that the arsenic compounds were dissolved in an aqueous growth medium containing microbial cells, nutrients and citric acid buffers. The retention volumes were not substantially altered in comparison to the distilled water matrix. For instance, distilled water and growth medium with DSMA had retention volumes ($\pm$ standard deviations) of 17.87 $\pm$ 0.21 ml and 16.97 $\pm$ 0.17 ml, respectively.

The retention times for the various arsenic compounds were found to lose resolution after the columns had been in use for approximately one month. The efficiencies of the SAX and SCX columns decreased substantially. Neither flushing the columns with a variety of organic solvent combinations (*e.g.* methanol, dichloromethane, acetonitrile, hexane) nor washing with 0.1 *M* hydrochloric acid returned the columns to their original efficiencies. Periodic replacement of the ion-exchange columns was necessary to insure maximum performance. The useful life of a column is extended when a small pre-column with comparable packing is placed before the analytical column[10]. Flushing the columns with methanol after a run and keeping them filled with methanol between runs insures reproducible retention times over many months.

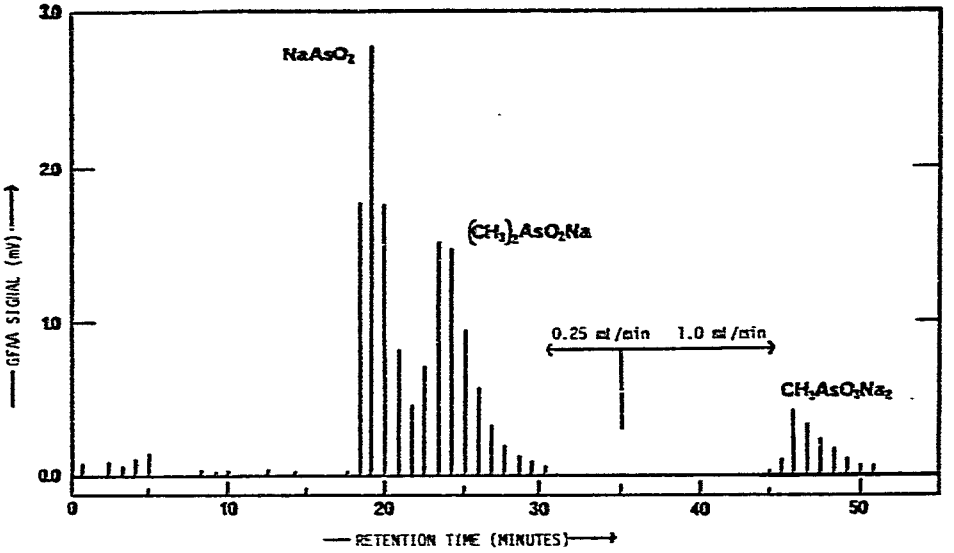

Fig. 2. Separation of sodium arsenite, SDMA and DSMA by the HPLC–GFAA technique (Perkin-Elmer GFAA) employing a SAX column: Altex LiChrosorb SAX column, 25 cm × 3.2 mm I.D., 10-μm particles; 20-μl sample containing 2 ng arsenic of each compound placed on column; mobile phase 0.030 *M* ammonium acetate–0.045 *M* acetic acid, pH 4.43; 20 μl of column effluent injected into graphite cuvette every 45 sec; no background correction.

TABLE I

RESULTS OF HPLC–GFAA SPECIATION EXPERIMENTS* WITH AND WITHOUT D_2 BACKGROUND CORRECTION FOR SOLUTIONS PREPARED BY DISSOLVING SODIUM ARSENITE AND SODIUM DIMETHYLARSINATE IN WATER OR 0.05 *M* AQUEOUS CITRIC ACID SOLUTION

Arsenic compound	*GFAA Signal, peak areas ($\mu V \cdot sec \times 10^{-3}$)*			
	D_2 off		*D_2 on*	
	0.05 M Citric acid	*Water*	*0.05 M Citric acid*	*Water*
$(CH_3)_2AsO_2Na$ (180 ng As/20 μl)	99.81	104.2	72.36	68.42
	101.2	104.0	70.86	65.63
$NaAsO_2$ (20 ng As/20 μl)	15.80	16.63	5.177	2.386
	17.52	15.80	5.140	2.992

* The mixture of $(CH_3)_2AsO_2Na$ and $NaAsO_2$ was separated on the SAX column with 0.030 *M* ammonium acetate–0.045 *M* acetic acid at a flow-rate of 0.25 ml/min. The sample volume was 20 μl.

Separations on SCX columns

Because arsenate was too strongly retained on the SAX column, a SCX column was employed to separate arsenate and arsenite with 0.030 *M* ammonium acetate–0.045 *M* acetic acid as the mobile phase (Fig. 3). Dimethylarsinate signals are located between the arsenate and arsenite signals with rather poor resolution. Addition of DSMA to a solution of these three compounds caused all peaks in the chromatogram to be poorly resolved. The separating ability of the cation-exchange column is not

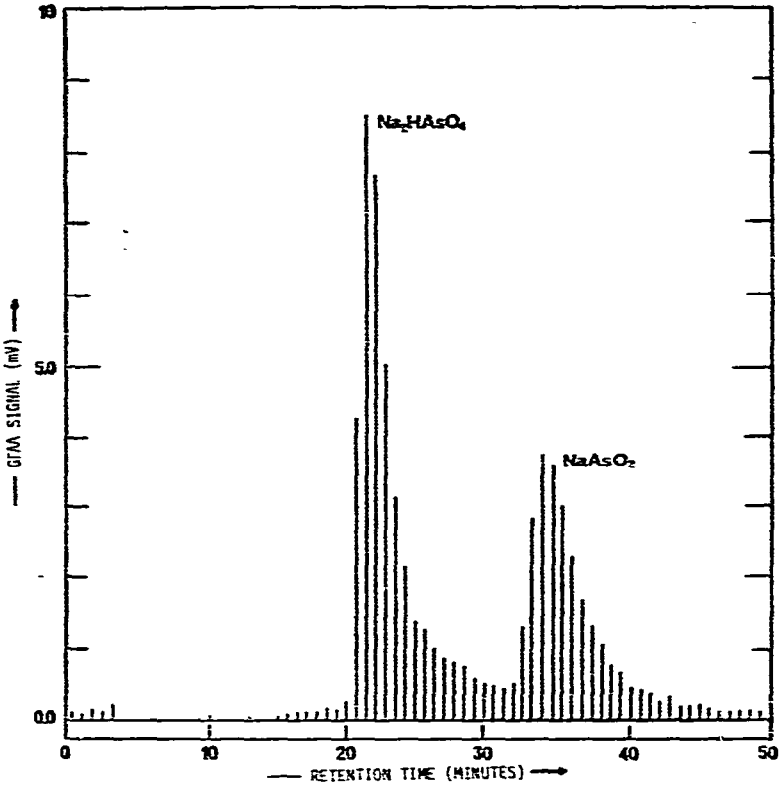

Fig. 3. Separation of disodium hydrogen arsenate and sodium arsenite by the HPLC–GFAA technique (Perkin-Elmer GFAA) employing a SCX column: Altex SCX column, 25 cm × 3.2 mm I.D., 10-μm particles; 20-μl sample containing 100 ng arsenic of each arsenic compound placed on column; mobile phase 0.0375 *M* ammonium acetate–0.0375 *M* acetic acid; flow-rate 0.15 ml/min; 20 μl of column effluent injected into graphite cuvette every 40 sec; no background correction.

associated with the cation-exchange function. The column acts by adsorbing the arsenic compounds on the organic backbone of the resin.

Separations on C_{18} reversed-phase columns

A C_{18} reversed-phase column can be used to separate ionic species provided the anions are paired with a suitable lipophilic positive counter-ion to produce a rather hydrophobic ion pair. Tetrabutylammonium phosphate (TBAP) at 0.005 *M* concentration in the appropriate mobile phase has been recommended as the reagent for such paired-ion chromatography. Arsenate and arsenite migrated almost with the solvent front through a C_{18} reversed-phase column with water–methanol (95:5) at pH 7.3 as the mobile phase in the absence of TBAP (Fig. 4a) but were completely separated in the presence of TBAP (0.005 *M*) with retention times of 19 min and 11 min for arsenate and arsenite, respectively (Fig. 4b). When a mixture of arsenate, arsenite, DMAA and MAA was chromatographed with 0.005 *M* TBAP in pure water, as the mobile phase, which should increase the retention time of lipophilic ion pairs,

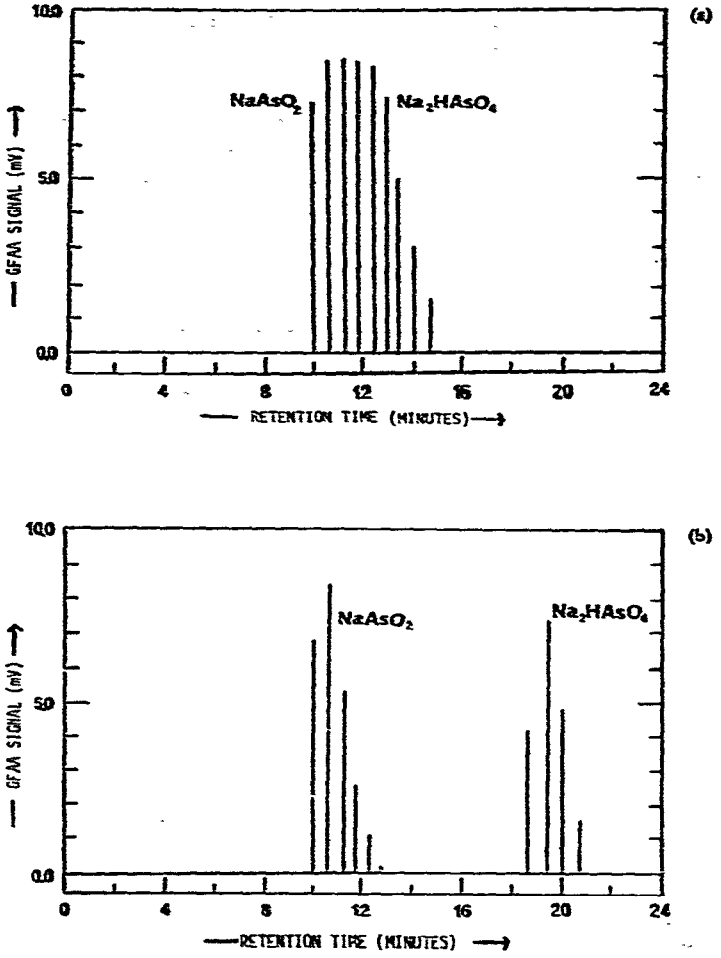

Fig. 4. Separation of disodium hydrogen arsenate and sodium arsenite by the HPLC–GFAA technique (Hitachi-Zeeman GFAA) employing a C_{18} reversed-phase column: Waters Assoc. μBondapak C_{18} column, 30 cm × 4.0 mm I.D., 10-μm particles; 40-μl sample containing 4.5 μg arsenate and 5.0 μg arsenite placed on column; flow-rate 0.5 ml/min; 40 μl of column effluent injected into graphite cuvette every 30 sec; mobile phase water–methanol (95:5), pH 7.3 (Fig. 4a); water–methanol (95:5) 0.005 *M* with respect to tetrabutylammonium ion, pH adjusted to 7.3 with phosphoric acid (Fig. 4b).

the peaks caused by arsenite and DMAA overlapped. The MAA signals are located under these conditions between those belonging to arsenite and arsenate.

A much better separation is achieved with tetraheptylammonium nitrate (THAN). Utilizing water–methanol (75:25) of pH 7.6 saturated with THAN as the mobile phase all four arsenic compounds are satisfactorily separated (Fig. 5). Arsenite is eluted almost with the solvent front, followed by DMAA, MAA and arsenate. The retention times of these compounds increase with increasing pK_a values of the arsenic acids. Arsenous acid ($pK_a \approx 10$) remains undissociated under these conditions, does not pair up with the tetraheptylammonium cation, retains its polar character and is,

therefore, not adsorbed onto the organic, reversed-phase column material. The weak acid, DMAA (p*K* 6.3) owes its retention with a retention time of 10 min to the two methyl groups rather than to its presence in solution as the tetraheptylammonium dimethylarsinate ion pair. The moderately acidic MAA (p*K* 3.6) is very likely combined with the ammonium ion and adsorbed by the column material as the tetraheptylammonium methylarsonate resulting in a retention time of 23 min. Arsenic acid with a p*K* value of 2.2, the most acidic compound among the four arsenic derivatives investigated, is strongly retained on the column head probably as the ion pair $[R_4N]_2^+ HAsO_4^{2-}$. The mobile phase must be changed to pure methanol to elute the arsenate.

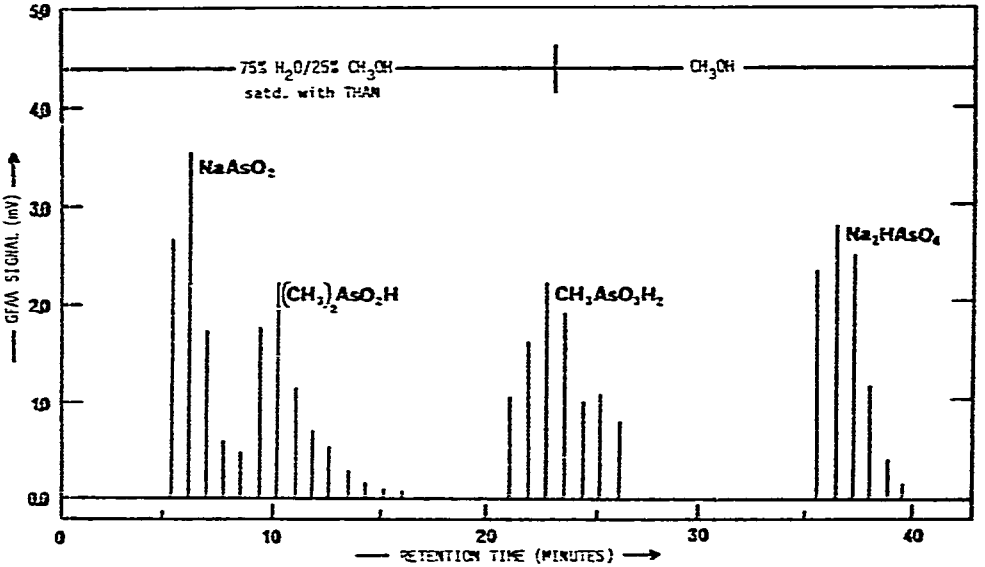

Fig. 5. Separation of sodium arsenite, DMAA, MAA and disodium hydrogen arsenate by the HPLC–GFAA technique (Hitachi-Zeeman GFAA) employing a C_{18} reversed-phase column: Altex Li-Chrosorb RP-18 column, 25 cm × 4.6 mm I.D., 10-μm particles, 100-μl sample containing 500 ng of each arsenic compound placed on column; mobile phase water–methanol (75:25), pH 7.6, saturated with tetraheptylammonium nitrate followed by pure methanol; flow-rate 1.0 ml/min; 40 μl of column effluent injected into graphite cuvette every 45 sec.

Quantitative determination of arsenic compounds

The GFAA signals produced by the arsenic compounds eluting from the columns can be quantitated either by calculating the area under the curves obtained by connecting the maxima of the GFAA signals or by summing the heights of the signals corresponding to a particular compound. In Fig. 6 the GFAA signal peak areas for arsenite, SDMA and DSMA (SAX column, Perkin-Elmer GFAA) are plotted against the amount of arsenic. The calibration curves constructed for the range 0.1–20 ng arsenic showed a linear dependence of the GFAA peak areas obtained as described earlier[6] on the amount of arsenic in the range 0.1–10 ng arsenic. Amounts of arsenic in excess of 10 ng gave peak areas much smaller than the values extrapolated from the lower range of the calibration curve.

Sodium arsenite, disodium hydrogen arsenate, MAA and DMAA can be

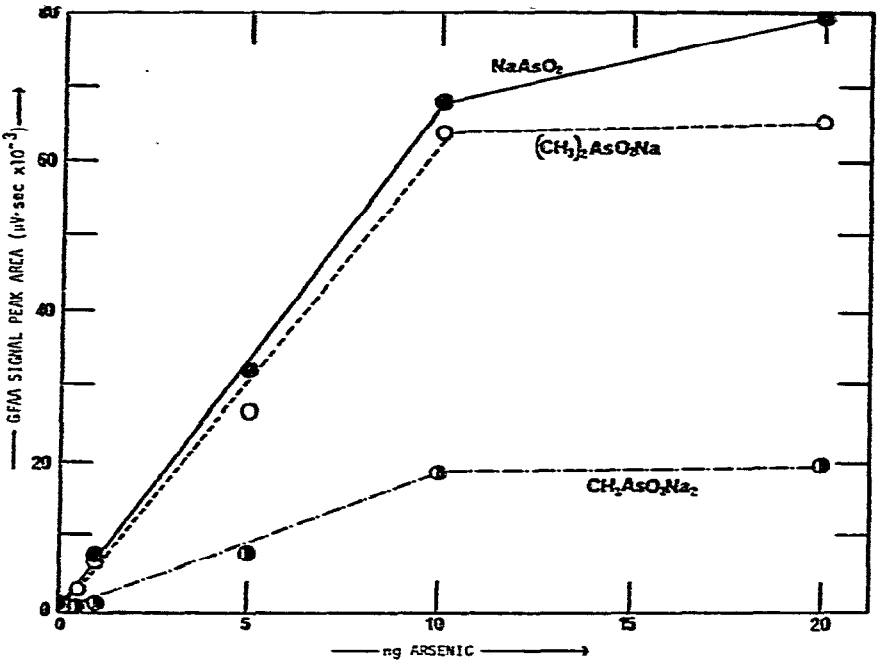

Fig. 6. HPLC-GFAA (Perkin-Elmer GFAA) Calibration curves for sodium arsenite (●), SDMA (○) and DSMA (◑): Altex LiChrosorb SAX column; 20-μl samples containing equal amounts of arsenic of each of the three compounds placed on column; mobile phase 0.030 *M* ammonium acetate–0.045 *M* acetic acid, pH 4.43; flow-rates as in Fig. 2; 20 μl of column effluent injected into graphite cuvette every 45 sec; no background correction.

separated and simultaneously quantitated employing a C_{18} reversed-phase column (Hitachi-Zeeman GFAA). The sum of the peak heights is linearly dependent on the amount of arsenic up to 500 ng arsenic with a change of slope of the calibration curve at 250 ng arsenic for all arsenic compounds investigated except arsenate. The calibration curves are probably linear over a wider range than indicated in Fig. 7. Chromatography of sodium arsenite in amounts varying from 20 to 1000 ng arsenic and summing the peak heights of the GFAA signals allowed the construction of Fig. 8. The slope change in the calibration curve again occurred at 200 ng arsenic. An example of a calibration curve for arsenite and arsenate with background correction (Perkin-Elmer GFAA) is presented in Fig. 9.

A comparison of two methods to convert the GFAA signal clusters, corresponding to a particular arsenic compound, to numerical values to be plotted against the amounts of arsenic proved that the summation of peak heights produces the same results as the determination of the areas under the signal clusters. A pooled regression analysis for the typical data of 42 determinations of sodium arsenite, SDMA and DSMA at varying concentrations, processed according to the "sum of peak heights" and "peak cluster area" methods, gave a correlation coefficient of 0.987. Summation of the peak heights, which is easier and faster than calculation of peak cluster areas is, therefore, the method of choice.

An intercept of the calibration curves close to zero as shown in Figs. 6–8 is

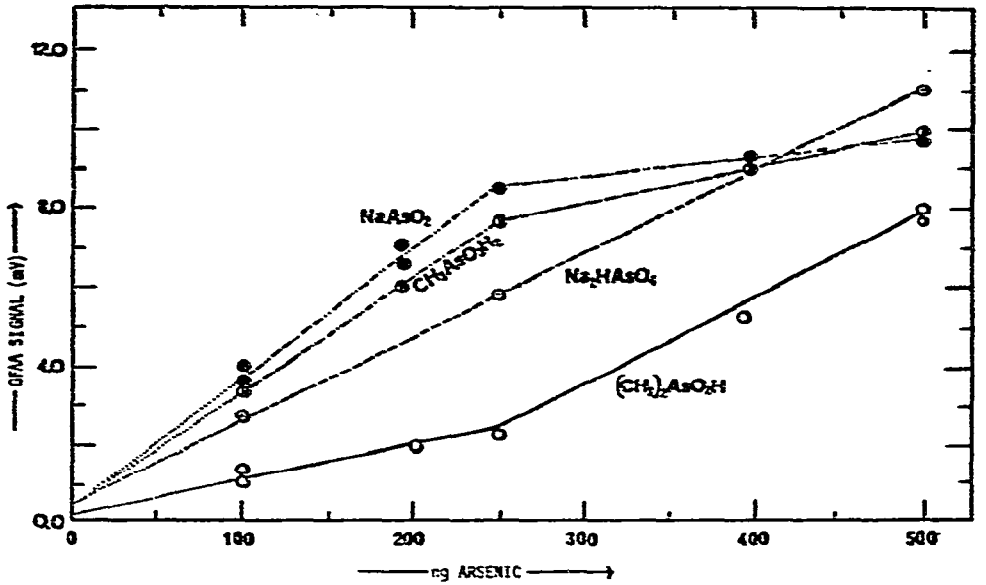

Fig. 7. HPLC-GFAA (Hitachi-Zeeman GFAA) calibration curves for sodium arsenite (●), DMAA (○), MAA (◑) and disodium hydrogen arsenate (⊙) employing a C_{18} reversed-phase column: Altex LiChrosorb RP-18 column; 20–100-μl samples containing equal amounts of arsenic of each compound placed on column; mobile phase water–methanol (75:25), pH 7.6, saturated with tetraheptylammonium nitrate followed by pure methanol (see Fig. 5); flow-rate 1.0 ml/min; 40 μl of column effluent injected into graphite cuvette every 45 sec.

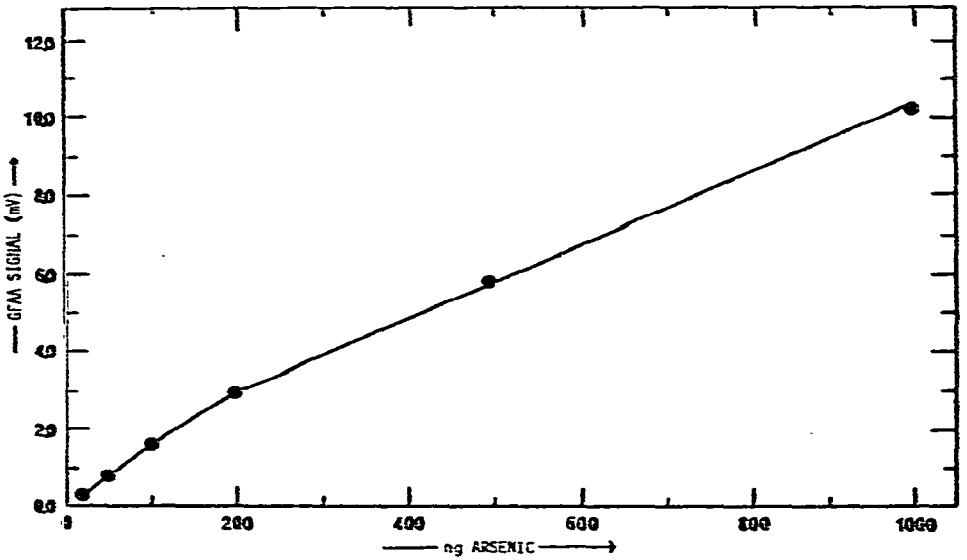

Fig. 8. HPLC-GFAA (Hitachi-Zeeman GFAA) calibration curve for sodium arsenite employing a C_{18} reversed-phase column: Altex LiChrosorb RP-18 column; 10–50-μl samples of 2 ppm or 20 ppm arsenic (arsenite) solution placed on column; mobile phase water–methanol (80:20), pH 7.4, saturated with tetraheptylammonium nitrate; flow-rate 0.5 ml/min; 40 μl of column effluent injected into graphite cuvette every 45 sec.

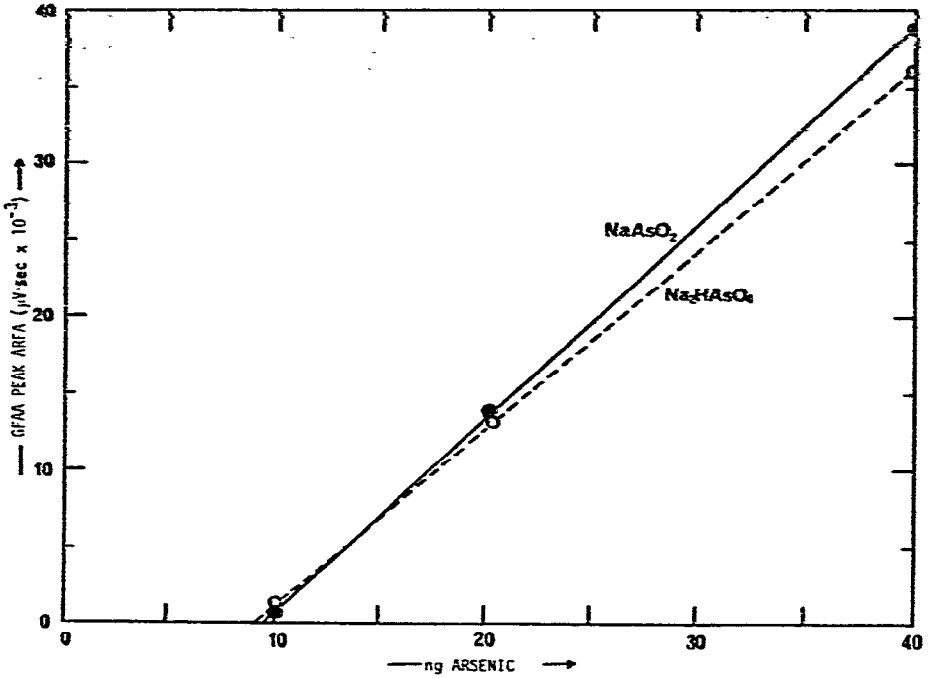

Fig. 9. HPLC–GFAA (Perkin-Elmer GFAA) calibration curve for sodium arsenite and disodium hydrogen arsenate employing a SCX column: Altex SCX column; 100 μl sample containing equal amounts of arsenic of each of the compounds placed on column; mobile phase 0.0375 *M* ammonium acetate–0.0375 *M* acetic acid; flow-rate 0.15 ml/min; 20 μl of column effluent injected into graphite cuvette every 40 sec; with D_2-background correction.

observed with the Perkin-Elmer GFAA as detector only in the absence of background correction. In the presense of background correction the intercept becomes negative with a sensitivity cut-off at 10 ng arsenic as shown in Fig. 9 for sodium arsenite and disodium hydrogen arsenate. Several reasons for this behavior of the pulsed GFAA detector are given in ref. 6.

The sensitivity and the general response of the GFAA instruments is rather strongly dependent on the characteristics of the graphite cuvettes and other parts of the furnace system. The cuvettes, for instance, tend to deteriorate with use and reduce the sensitivity. A new cuvette does not necessarily produce the same response as one subjected to a number of analysis cycles. This behavior necessitates the frequent use of standards. The Perkin-Elmer GFAA operating without background correction is presently more sensitive as an HPLC detector than the Hitachi-Zeeman system, but the operator must verify the atomic origin of signals. As little as 0.1 ng arsenic (Fig. 6) was detected by the Perkin-Elmer instrument under ideal conditions, which are difficult to reproduce. However, several nanograms of arsenic, passed through the chromatographic system, can routinely be quantitated in the present stage of development of the HPLC–GFAA system. On the other hand, the Hitachi-Zeeman GFAA serving as detector for the effluent from a C_{18} reversed-phase column is, for instance, capable of detecting several nanograms of arsenic under ideal conditions (Fig. 8) and approxi-

mately 10 ng arsenic of sodium arsenite in the presence of DMAA. The determinations are accurate within ±5–10%, and are intrinsically background corrected. The causes of the changes in slopes of the calibration curves are not clear yet and need to be investigated further.

The various arsenic compounds produce different GFAA responses for the same amount of arsenic (Fig. 10). As much as a two-fold difference in intrinsic GFAA sensitivity was observed between DMAA and MAA as well as between their sodium salts. The less volatile species give a more intense signal than the more volatile compounds. Atomization processes different for each of the compounds and a higher loss of the more volatile derivatives from the cuvette during atomization by thermal transport are likely causes for the observed behavior. The sensitivity of the GFAA instrument increases with increasing atomization temperature for all arsenic compounds tested (Fig. 10). These results emphasize the necessity of close control of the atomization step and of construction of calibration curves for each compound to be studied.

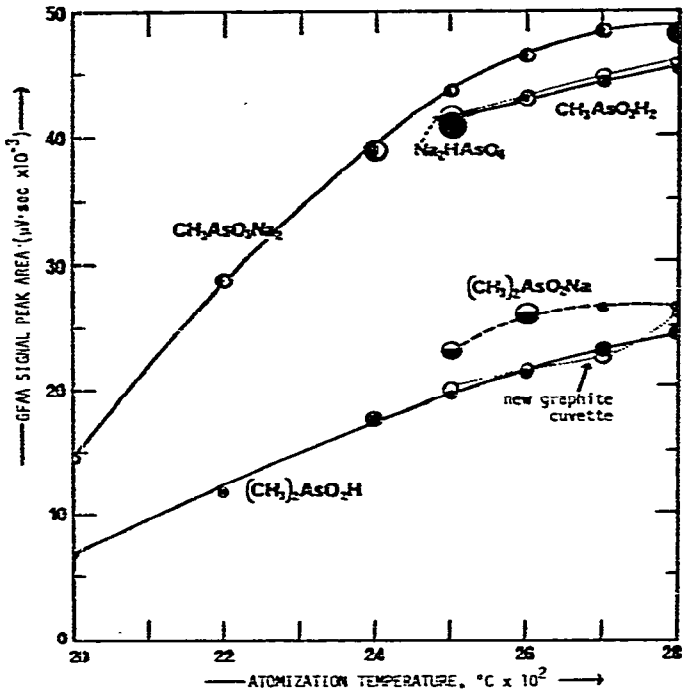

Fig. 10. Dependence of GFAA peak areas (Perkin-Elmer GFAA) on the atomization temperature and the chemical nature of the arsenic compound: 20-μl sample containing 10 ng arsenic of the desired arsenic compound in distilled water manually injected into the graphite cuvette; 10 sec drying at 150°; 5 sec charing at 200°; 10 sec atomization at indicated temperature.

Speciation of arsenic compounds in soil samples and drinking water by the HPLC–GFAA technique

Employing the HPLC separation schemes for arsenic compounds, several soil and drinking water samples were analyzed for arsenate, arsenite, MAA and DMAA.

Each soil sample had been treated in the field with either sodium arsenite, SDMA or MSMA. After approximately one year of exposure to environmental

conditions the remaining total arsenic concentrations obtained by acid digestion and GFAA analysis ranged from 30.4 μg/g to 70.4 μg/g*. Extraction of the water-soluble arsenic compounds with 1.0 *M* NH_4Cl (ref. 11) or 0.075 *M* ammonium acetate in place of NH_4Cl produced solutions with less than 2 ng As/20 μl (< 3 μg As/g in dry soil). Chromatography of 100 μl of these solutions on SAX and SCX columns without background correction gave a high background but no GFAA signals (Perkin-Elmer GFAA) attributable to arsenic compounds.

Detectable amounts of arsenic were dissolved from the soils by a solution containing NH_4F (0.5 *M*) and NaOH (0.1 *M*), which extracts most of the arsenic bound to iron and aluminum components in the soil*. Chromatography of the extracts on SCX columns using a 100-μl sample volume injected into the graphite cuvette, indicated that arsenate was present at levels of 23 ng/100 μl (7.1 μg/g), 21 ng/100 μl (6.5 μg/g) and 0 ng/100 μl (<3 μg/g) arsenic in the samples of the extracts from the soils treated with dimethylarsinate, arsenite and methylarsonate, respectively. The values in parentheses are the estimated concentrations of arsenic (as arsenate) in the soils on a dry weight basis. The speciated arsenate signal produced by the extractant accounted for less than 10% of the total arsenic obtained by complete digestion of the soil. No arsenite signals were observed indicating that the arsenite concentrations in the extracts were <10 ng/100 μl and <3 μg/g in the dry soil. No arsenite or arsenate leachable from soil treated with methylarsonate could be detected with a detection limit of <3 μg/g. The 0.5 *M* NH_4F–0.1 *M* NaOH extractant removed approximately 10% of the total arsenic in the soil, which appears to have been bound as arsenate to the iron and aluminium compounds in the soil.

These results prove that the HPLC–GFAA technique can be used to determine arsenic compounds in soil extracts. The required analytical separation and sensitivity are available, but the methods to extract all of the arsenic compounds from the soil without chemical alteration have not been developed yet.

Drinking water samples which contained arsenite, arsenate, methylarsonate and dimethylarsinate were not available. Therefore, a tap water sample with 315 ppm sodium, 316 ppm bicarbonate and 98 ppm chloride was spiked with the arsenic compounds to produce a solution containing 5.00 ppm arsenic of each compound. Immediate chromatography of 100 μl of this sample, employing the Hitachi-Zeeman GFAA as arsenic-specific detector and calibration curves for each compound, produced results within 5% of the theoretical values.

CONCLUSION

HPLC–GFAA systems have been shown to be applicable to the qualitative and quantitative determination of arsenite, arsenate, methylarsonate and dimethylarsinate in the ppb range with detection limits approaching 5 ppb under favorable conditions. The GFAA detector responds only to a specific element and is better suited for trace element compound determinations than UV detectors, which require the presence of an UV-absorbing group in the molecule containing the trace element (Fig. 1). When all four compounds are present in solution, arsenite, methylarsonate and dimethylarsinate can be separated and quantitatively determined at concentrations as low as

* These analyses were obtained from E. Woolson (USDA) as part of an NBS–USDA collaborative effort in the speciation of arsenic compounds in soils.

5 ppb even in rather complex matrices employing a SAX column. Arsenate and arsenite can be determined using a SCX column provided methylarsonate is not present. All four compounds can be separated and quantitated on a C_{18} reversed-phase column. These separation schemes are applicable to the determination of arsenic compounds in soil extracts and drinking water samples. The separations reported in this paper very likely can be improved by application of columns tailored to arsenic compounds. As an example of column materials development the preparation of reversed-phase cation- and anion-exchange resins is mentioned. These columns were used to separate heterocyclic nitrogen bases and ribonucleoside phosphates[12] and should be applicable to the separation of arsenic compounds. Recently the use of a proprietary special anion-exchange column for the separation of arsenic compounds was reported[13]. Improved column design will make the HPLC–GFAA technique even better suited for the speciation of trace element compounds in a variety of matrices. The work with arsenic compounds clearly shows the great potential of these systems in the area of environmental trace element chemistry, in the field of physiological chemistry and in trace-element related nutritional studies.

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