Arsenic Speciation in Humans and Food Products: A Review

L. Benramdane[1,2], **F. Bressolle**[3]*, and **J.J. Vallon**[1,2]

[1]Laboratoire de Biochimie, Pharmacotoxicologie et Analyse des traces, Hôpital E. Herriot, Lyon, France; [2]Laboratoire de Chimie Analytique III, Faculté de Pharmacie, Lyon, France; and [3]Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, Montpellier, France

Abstract

Although acute intoxication has become rare, arsenic (As) is still a dangerous pollution agent for industrial workers and people living in the vicinity of emission sources. In humans, only inorganic As is toxic; organic forms present in large amounts in the environment are nontoxic. It is therefore important to be able to differentiate one group from the other using appropriate speciation methods. The authors review the present knowledge of the distribution of As in humans and food products. The three steps of the speciation methods (sample preparation, species separation, and detection) are described. For liquid samples, a clean-up step (C_{18} cartridge extraction, dilution, or freezing) is necessary to eliminate proteins and salts from the matrix. For solid organic samples, the first step consists of the digestion of tissues followed by solvent extraction sometimes coupled with a C_{18} extraction. The separation of As species is accomplished by different high-performance liquid chromatography (HPLC) methods (ion-exchange, ion-pairing, and micellar liquid chromatography). The detection methods are compatible with HPLC and are able to detect As species in the microgram-per-liter range. Inductively coupled plasma (ICP) atomic emission spectrometry is more frequently used, but suffers from interference by organic solvents in the mobile phases. Atomic absorption spectrometry methods give sensitivities of the same order. ICP-mass spectrometry has the advantage of specificity and can be 100- to 1000-fold more sensitive than previous methods.

Introduction

For many years, arsenic exposure has been associated with various disease states (cancer, cardiovascular disease, neurological syndromes). Numerous fatal intoxications have been reported (1–4). Recently, fatalities due to arsenic have become rare; drinking water, sometimes contaminated by inorganic arsenical species of natural or industrial origin, is the principal source of these fatal intoxications (5–7). In some rare cases, suicide by inorganic arsenicals is observed (8).

In most cases, arsenic exposure is evaluated by the total arsenic concentration measurement in biological media. However, several studies provided proof that a unique consumption of seafoods can give rise to an important increase in urinary total arsenic within the 10 h of ingestion (9,10). This increased excretion is without any consequence on health, because absorbed arsenic is under organic forms without any toxic effect (9,11,12). These facts demonstrate the absolute necessity of determining individual concentrations of every arsenical species either in biological or in environmental media in order to differentiate toxic from nontoxic species. This differentiation, called speciation, can separate inorganic arsenic, namely arsenite (As[III]) and arseniate (As[V]), and its metabolites monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) from nontoxic arsenicals of marine food origin, namely arsenobetaïne (Asbet) and arsenocholine (Aschol).

In acute intoxications following the ingestion of large quantities of inorganic arsenicals, the major symptoms are gastrointestinal (nausea, vomiting, abdominal cramps, and diarrhea that may be blood stained), sometimes followed by cardiovascular collapse and death (8,13). If intoxication is not fatal, the patient suffers from a central and peripheral neuropathy, hepatic failure and vascular lesions giving rise to gangrene of the extremities, and myocardial infarction (8,14). The lethal dose is probably between 100 and 200 mg of arsenious acid for an adult individual, although people have survived larger doses (15). This dose varies with the arsenic species implicated. The 50% lethal dose values (LD 50 for oral administration to mice, in milligrams per kilogram) are as follows: arsin, 3; arsenite, 14; arseniate, 20; MMA, 700–1800; DMA, 700–2600; Asbet and Aschol, > 10,000 (16). During the last decades, the diminution of acute intoxications by arsenic was originating from the very significant decrease in the use and worldly production of arsenicals (77,000 tons in 1970 to 29,000 in 1980) (17). However, arsenic use is still important in industry (smelting, glass making, and semiconductor manufacture), agricultural and wood preservation, and

* Author to whom correspondence should be addressed.

numerous mining operations and industries (9,14,18). Arsenic, being very ubiquitous, is distributed in every compartment of the environment (air, water, and soil) as soon as it is emitted. Therefore, it is a dangerous pollution agent for industrial workers and inhabitants living in the vicinity of emitting sources.

Chronic intoxications are observed following continuous exposure to even very low doses of inorganic arsenic during a few years. Environmental arsenic exposure is associated with general effects (chronic weakness, loss of weight, debility, loss of appetite, anemia apparently due to bone marrow suppression, chronic renal and liver damage, vascular effects such as gangrene, and myocardial infarction), dermatological manifestations (hyperpigmentation and hyperkeratosis), and effects on the nervous system (paresthesis, muscle cramp, ataxia, and lack of coordination) (13,15,19). After some years of exposure, arsenic causes a skin cancer due to the ingestion of soluble inorganic arsenicals, lung cancer due to inhalation (smelter workers), and many other types of malignant neoplasms (tumors of stomach, esophagus, and urinary bladder) (15,20–23).

Because of its ubiquity in the environment, arsenic is present in biological media of animals and plants, even without any intoxication or seafood consumption. Its normal concentrations vary according to the medium concerned. In blood, total arsenic varies from 1.5 to 2.5 µg/L (15). Buratti et al. (10) measured the blood concentration in 148 normal individuals and found an average value of 5.1 µg/L. Concentrations in hair vary from 0.25 to 0.88 µg/L (24); in urine, average concentrations are between 20 and 50 µg/L (11,24).

In toxicological analysis, the urine medium is most often used as a proof of intoxication, because urinary excretion is the principal form of arsenic elimination (9,10,15). Moreover, a good correlation is observed between absorption and urinary excretion. Blood serum is also used, especially to detect an accumulation risk of arsenic species in patients with chronic renal diseases with or without hemodialysis (25,26) and in occupationally exposed people to inorganic arsenic in the workplace (9,14). As a general rule, total urinary As concentration is used to prove arsenic exposure, but the measurement of total As may not always be appropriate for the biological monitoring of occupationally exposed workers. Indeed, numerous studies have shown that total urinary arsenic can show an important rise within the 10 h following seafood ingestion (9,10). Moreover, these elevated levels are without any consequence on health, because arsenic of dietary origin is exclusively in organic, and therefore nontoxic, forms (9,11,12).These observations are in favor of the selective determination of every species present (that is, speciation of arsenicals). The species of concern are the toxic inorganic As(III) and As(V) species and their metabolites (MMA and DMA) together with nontoxic organic species of dietary origin (Asbet and Aschol).

Arsenic species of dietary origin are mainly found in marine foods with high concentrations. Seaweeds used in human foods (nori, wakame, kombu) have a content in total arsenic between 3 and 200 µg/g; content in crustaceans is situated between 10 and 100 µg/g. In fish, contents vary according to the species of fish concerned (11,27); average concentrations vary between 5 and 100 µg/g (27,28), whereas elevated values of 100 to 250 µg/g are found in the last species of the food chain such as the conger or the dogfish (27–29). In flat fishes (plaice, sole, flet), mean values are between 10 and 60 µg/g (27). Nevertheless, it has now been demonstrated that these important concentrations in seafood cause no damage to health, because 80–95% of arsenic is in the organic form (Asbet, Aschol, arsenosugars, and arsenolipids) (11,12,29–31). Beauchemin et al. (29), in a study of arsenic speciation in dogfish muscle, concluded that among the 94% of As extracted (35 µg/g of dry weight), 84% are arsenobetaine and 4% are As(III), As(V), MMA, and Aschol. The remaining 6% are other undetermined chemical species, probably lipid-bound (arsenolipids) because of their solubility in chloroform. Nevertheless, Goessler et al. (11), in a recent study, succeeded in identifying an arseno-ribose dimethylarsinoriboside that accounts for most of the arsenic present in a seaweed (*Hormosira bonskii*). In a gastropod (*Austrocochlea constricta*) that consumes the seaweed, total As (74.4 µg/g of dry weight) is composed of several arsenical species, mainly arsenobetaïn together with minor species, among which are As(V), DMA, Aschol, arsenoribose, tetramethylarsonium, and As(III). On the other hand, in another gastropod (*Morula marginalba*) which consumes *Austrocochlea constricta*, 96% of total extracted As (233 µg/g of dry weight) is arsenobetaïne, and the remaining 4% is arsenocholine. These results show that arsenobetaïne is the main end-product of the arsenic metabolism in the food chain and that consumption of the last marine species of the food chain is less hazardous for inorganic As absorption.

Because of its ubiquity, arsenic is naturally found in all waters, with an average concentration approaching 1 to 2 µg/L (15). The arsenic cycle within the groundwater compartment has important implications in human toxicology (32). The weathering of arsenic-bearing minerals is generally the most important source of inorganic arsenic in groundwater (33). In surface water, anthropogenic sources can add substantially to the arsenic content. Inorganic As comes from minerals, industrial discharges, and insecticides; organic forms have their origin in industrial discharges, insecticides, and biological actions on inorganic As (phytoplankton). The As concentration in most drinking waters seldom exceeds 10 µg/L, although values as high as 135 µg/L have been found in India (33). For public health reasons, several government and international organizations (34–37) have prescribed a permissible total As concentration between 10 and 50 µg/L in potable water. Although all regulations relate only to the total water amount of As, the speciation of As is important to consider in differentiating toxic inorganic As from the nontoxic organic species.

In humans, very few studies have been done on the urinary speciation of As. Buratti et al. (10) showed, in a study of 148 normal subjects not exposed to As and without any As marine absorption, that total urinary concentration is 17.2 µg/L, of which 1.9 µg/L is inorganic (As[III] + As[V]), 1.9 µg/L is MMA, 2.1 µg/L is DMA, and the other part is undetermined forms. In the same population, a few days following marine food consumption, total As in urine reached 132.3 µg/L, whereas inorganic As, MMA, and DMA were unchanged; only nontoxic organic As was increased. Arbouine and Wilson (9), in an other study on healthy volunteers, found that only inorganic As remains constant following fish absorption. In contrast, Lowell and Farmer (8) found that the total urinary As in workers chron-

ically exposed to inorganic As is increased up to some hundreds of micrograms per liter with the following distribution of the different species: As(V), 3.5%; As(III), 12.5%; MMA, 16%; DMA, 66.5%. The important increase in DMA compared with MMA is the sign of a chronic intoxication by inorganic As (32). In seafood, as well as in human, the species generally studied are As(III), As(V), DMA, MMA, Asbet, and Aschol. Some other methods determine tetramethylarsonium and arsenosugars present in marine foods (11,31).

Therefore, As speciation appears a more and more important analytical step in water analysis, food and agricultural areas, or in human health (controls in the workplace or in patients with chronic renal disease). In this review, the research was limited to As speciation in human biological media, marine foods, and drinking waters.

Experimental

General considerations of As speciation

Speciation is generally accomplished in three steps: sample preparation, species separation, and detection. Sample preparations consist of the isolation of different species from the matrix, sometimes with a preconcentration effect. Indeed, biological samples are complex, and species to be analyzed are very diluted. Sample preparation is a very critical step necessary to efficiently solubilize the species without degradation. High-performance liquid chromatography (HPLC) is the method of choice, because solutes to be separated are neither volatile nor thermostable, and gas chromatography (GC) is applicable only after derivatization. Moreover, HPLC is easily coupled with several of the existing methods of detection. The classical detectors in HPLC (ultraviolet–visible or electrochemical detectors) are not applied in biology because of their poor selectivity and sensitivity for the determination of species present at low levels in a complex matrix. In these media, concentrations are at the nanogram-per-gram level for solid samples (seafood) and the microgram-per-liter level for liquid samples (serum, urine). The detection methods generally used are those of the elementary analysis. The most sensitive detection method is mass spectrometry (MS) with a plasma source as the source of ionisation (inductively coupled plasma, ICP). Nevertheless, ICP-MS is too expensive to acquire and monitor to be present in most laboratories. For these reasons, a number of works on speciation use less expensive detectors such as ICP-atomic absorption spectrometry (ICP-AAS), graphite furnace AAS (GFAAS), or quartz furnace AAS (QF-AAS).

Speciation in biological samples is always preceded by total arsenic determination. The sample is dry-ashed (31,33) or submitted to wet-ashing (34–36) or microwaves (27,37). With dry-ashing, the organic matter is decomposed under the conjugate action of heat and magnesium nitrate $MgOMg(NO_3)_2$ (32,33). In wet-ashing, the reagents are mixtures of strong acids (HNO_3–$HClO_4$, HNO_3–H_2SO_4) (35) with which H_2O_2 is sometimes associated (38,39). The detectors used are GFAAS (39–41), hydride generation (HG)-GFAAS (27,38), HG-ICP-AES (34), and ICP-MS (33,35,42). The general scheme of all the procedures used for As speciation in biological media is given in Figure 1.

Sample preparation for As speciation

This stage is very critical for several reasons; biological media are very complex matrices with a high concentration of organic matter and salts that can interfere with the determinations. They contain very diluted As species; sample preparation should therefore ensure the extraction and concentration of the species without denaturation, loss, or contamination.

Samples are either liquid (water, serum, urine, dialysate) or solid (animal or vegetal tissues). In view of the elimination of these matrices, various extraction–purification procedures (depending of the liquid or solid nature of the sample) are available.

Pretreatment of liquid samples

Serum and urine have a high salt and organic material content, and serious problems (lack of separation, peak splitting or broadening, and dramatic shortening of the column life) appear if they are directly injected into a HPLC system. The use of C_{18} cartridges to partially clean-up the urine prior to injection into HPLC columns greatly enhance the column life (52,53). However, some speciation methods for As are limited to a simple fivefold or tenfold dilution followed by a filtration step (54,55). Other methods use C_{18} cartridges for urine purification, but the elimination of salts is then incomplete and their high concentrations lead to poor chromatographic separations (53,56). Indeed, Heitkemper et al. (56), studying As speciation by ion exchange, observed peak broadening, retention time shifts, and baseline modifications when the samples were supplemented in sodium chloride. Moreover, in HPLC–ICP-MS, chlorides greatly interfere by forming a very intense peak caused by the polyatomic ion $^{40}Ar^{35}Cl^+$ with the same mass-to-charge ratio as the ^{75}As isotope. In this case, the chromatographic separation between the interfering chlorides and other species is possible, provided a dilution of urine is accomplished (53,57,58). The elimination of this interference is also possible with the use of a helium microwave plasma (argon is then eliminated) (59) or hydride generation (60). In a recent study, Lopez-Gonzalves et al. (61) proposed the purification of As species from urine by freezing (–15°C) samples diluted in absolute ethanol; at this temperature, salts and organic compounds of high molecular weight are precipitated, and the ethanol phase containing As species separates. After a further washing of the precipitate by ethanol, dry extracts are injected in the HPLC system consisting of a ion-exchange HPLC with HG-AAS detection (HPLC–MO-HG-AAS). Under these conditions, all interfering substances are eliminated (especially chlorides) as well as the organic matrix responsible for column damage.

Pretreatment of solid samples

Pretreatment of solid biological samples is much more complicated than for liquid samples and is generally accomplished in two steps: extraction followed by purification. The extraction step uses organic or hydro-organic solvents; in early methods, methanol followed by ether purification was used (62). Methanol–water (1:1) mixtures (63) and methanol–chloroform (2:1) extractions followed by reextraction with the methanol–water mixture were also used (29,46,51). The yield of extraction by the methanol–chloroform mixture is 94%, but reextraction with methanol–water decreases this yield to 88%. The difference corresponds to 6% of As still present in chloroform and probably

of lipidic nature (arsenolipids) (29). Another procedure which must be emphasized is the extraction of arsenobetaïne and arsenocholine by ion-pairing with hexanitrodiphenylamine (64). The enzymatic digestion of solid tissues has been proposed (51,65); tissues were digested by trypsin in order to extract the arsenic species, but the enzyme led to degradation of arsenobetaïn and dimethylarsinic acid. Nevertheless, Lamble et al. (65), with this method, recently found yields between 87 and 98%, but the enzymatic digestion necessitates a 6-h incubation time that makes this technique rather long. All these methods of extraction–purification have been applied to marine foods. For arsenosugars, a series of 5 reference materials (National Institute for Environmental Studies number 6 mussel tissue; NIST 1566 oyster tissue; and NRCC, TORT-1, and CRM 525 mussel tissues) have been used by Larsen (66) in an extraction using the ternary mixture water–methanol–chloroform. Alberti et al. (52) has

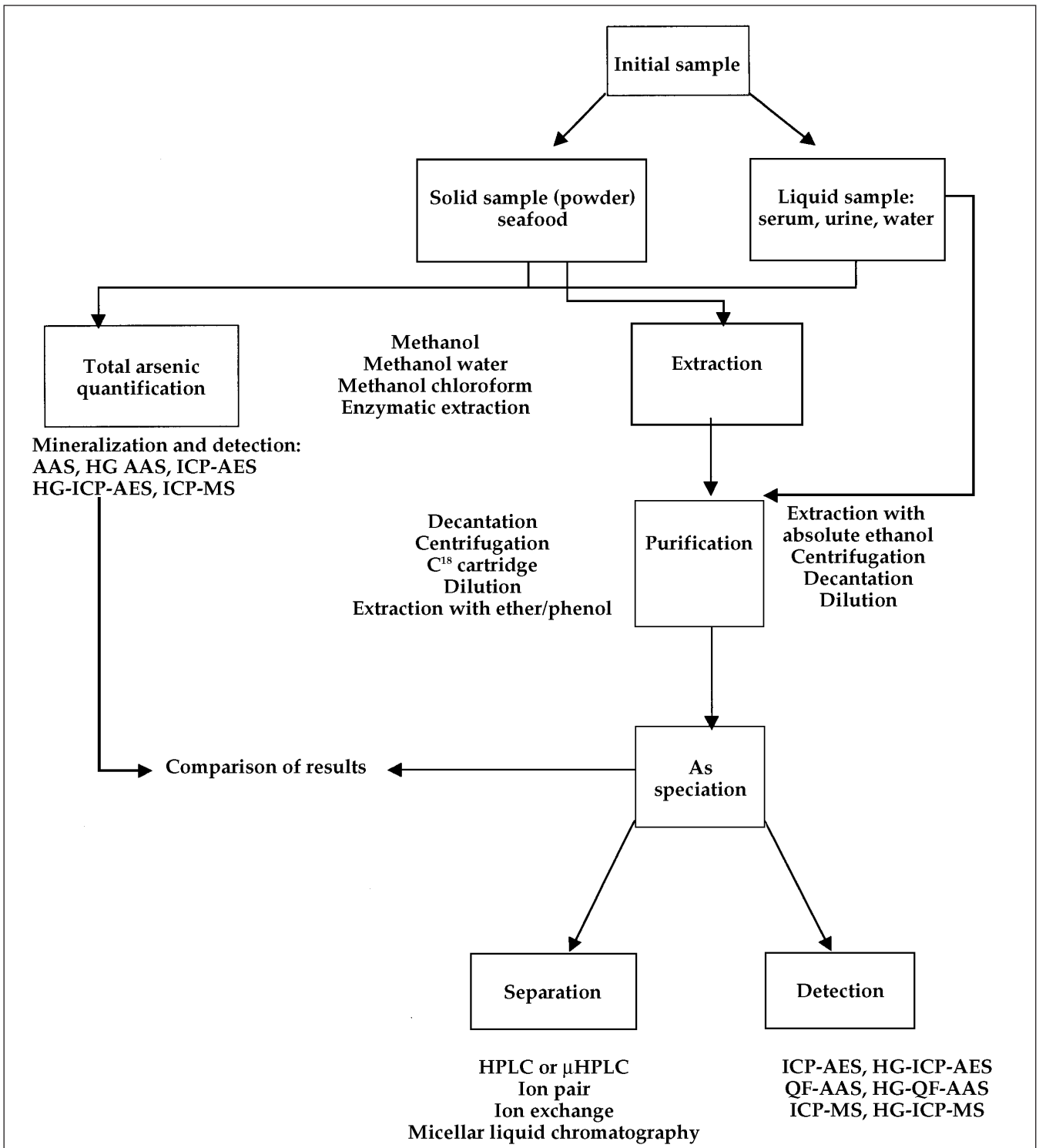


Figure 1. General scheme used for arsenic speciation in environmental and biological samples.

tested 3 extraction solvents (methanol–chloroform [1:1], methanol, and water–methanol in various ratios) by their action on a reference material (CRM 278 mussel tissue); the best mixture was found to be the water–methanol mixture in different ratios. The elimination of interfering substances and organic matter in the raw extract is affected by a solid–liquid purification on a C_{18} preparative cartridge without any loss. Elmoll et al. (53) compared 3 extraction methods applied to a ray extract: the extract was simply centrifuged, the same extract was purified through liquid–liquid extraction by an ether–phenol mixture, and the third part was extracted on a C_{18} cartridge. The anion-exchange chromatograms showed poor resolution of Asbet and Aschol when the raw extract was only centrifuged. The ether–phenol purification increased the resolution slightly but incompletely. Finally, C_{18} separation gave the best results, but the retention times were different than those for the standards, probably because of the high content of salts in the extracts. Therefore, the authors propose a 10- or 50-fold dilution of the raw extracts to obtain sufficient peak resolution; nevertheless, a very sensitive detector such as ICP-MS must then be used to ensure the accurate determination of analytes.

One can conclude that solid sample pretreatment is a very tedious step that must be studied with much care for every particular sample when marine foods are concerned (Table I).

Separation of arsenic species

HPLC with classical columns or microcolumns is the most frequent technique used for speciation. GC has been applied, but As species must be derivatized before separation (67–69). Capillary electrophoresis is an interesting recent technique, but the sensitivity of the detectors associated with it (ultraviolet [UV], conductimetry) is low, so that its application to speciation is rare (70).

The polarity of organic and inorganic compounds makes them amenable to both ion-exchange and reversed-phase separations. There are generally 6 As species to be analyzed: As(III), As(V), DMA, MMA, Asbet, and Aschol. Nevertheless, a number of methods for As speciation in biological media or water samples apply only to inorganic As determination (36,71). Other methods include an additional determination of DMA and MMA (10,57,72). Indeed, recent chromatographic speciation methods applied to biological media allow the determination of 6 or even more species, including the tetramethylarsonium ion and the arsenosugars (11,40).

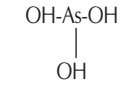
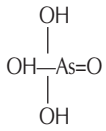
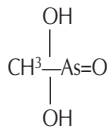
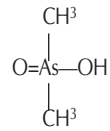
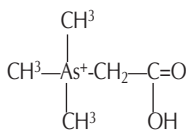
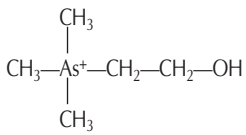
The physico-chemical properties of the principal species play an important role in the liquid chromatographic (LC) separation; inorganic species and their methylated forms are polar weak acids, Asbet is a weak acid with a permanent positive charge on the As atom, and Aschol has the same positive charge

Table I. Procedures Used for Arsenic Extraction and Purification in Biological Samples

Sample	Extraction procedure	Extraction yield (%)	Purification	Time of procedure	Side effect on the chromatographic separation	Reference
Certified reference Materials (CRM)						
Cod muscle CRM 422	methanol–water (1:1)	97,30	C_{18} cartridge	> 100 min	small resolution of Aschol and Asbet	44
Dogfish muscle DORM-1	methanol–water (1:1)	95,64	C_{18} cartridge	> 100 min	small resolution of Aschol and Asbet	44
Mussel tissue CRM 278	methanol–water (1:1)	54,24	C_{18} cartridge	> 100 min	small resolution of Aschol and Asbet	44
Ray, sole	methanol–water (1:1)	—	extraction with ether–phenol, C_{18} cartridge, dilution	> 120 min	decrease in resolution between Asbet and Aschol, shifted retention times	53
Seaweed Hormosira bonskii	methanol–water (9:1)	30			decrease in resolution	11
Gastropod Austrocochlea constricta	methanol–water (9:1)	74	centrifugation and filtration	> 14 h	decrease in resolution	11
Gastropod Morula maginalba	methanol–water (9:1)	95	centrifugation and filtration	> 14 h	decrease in resolution	11
Dogfish DORM-1	methanol–chloroform–water	94 with methanol–chloroform; decrease to 88 by methanol–water extraction	phenol ion-pair extraction and vacuum distillation sublimation	> 100 min (extraction)	separation destroyed, Asbet eluted for 2 peaks	29
Urine	absolute ethanol at –15°C	~ 100	centrifugation and evaporation of ethanol	2–5 h	any difference with standard sample	61
CRM of fish (DORM-1)	enzymatic extraction by trypsin	87	centrifugation, filtration	> 6 h	broadening of peak	65
CRM of fish (TORT-1)		98	centrifugation, filtration	> 6 h	broadening of peak	65

together with a primary alcohol function and a very low polarity in comparison with the other solutes. All these properties are the origin of difficulties in the choice of the chromatographic conditions (column, pH, and composition of the mobile phase) whatever the separation mode (ion exchange, ion pairs). Table II indicates that anionic As species (As[III], As[V], MMA, DMA) have very different acidity constants. This is an advantage in the separation of these solutes by ion-exchange or ion-pair partitioning. As(III) has an elevated pK_a value (9.2), so that it remains un-ionized in the pH range generally allowed (pH 2–8) for the common chromatographic stationary C_{18} bonded phases. In this pH range, the anionic species As(V), DMA, and MMA are retained on the column and well separated, whereas the As(III) un-ionized species elutes in the dead volume. In the anion exchange mode and in the same pH range of 2–8, As(III) is eluted with the same retention time as Aschol (cationic) and Asbet (either a cationic or a zwitterion, depending on the pH value). Morita et al. (73) succeeded in the resolution of 5 As species (As[III], As[V], DMA, MMA, Asbet, and Aschol) on a Nucleosil-N$(CH_3)_2$ anion-exchange column (Nagel, Hoerdt, France) using a neutral phosphate buffer as the mobile phase. At this neutral pH, Asbet and As(III) both remain in their neutral form so that hydrophobic interactions can be held and account for the differences in retention on this packing material. DMA elutes later than MMA, showing the affinity of the methyl groups of the As species towards the alkyl groups of the column packing.

Table II:. Formulas and pK_a of Principal Arsenic Species

Compound	Formula	pK_a
Arsenious acid As(III)		9.2
Arsenic acid As(V)		2.3 6.8 11.6
Monomethylarsonic acid (MMA)		3.6 8.2
Dimethylarsinic acid (DMA)		1.3 6.2
Arsenobetaine (Asbet)		2.2
Arsenocholine (Aschol)		—

In another connection, Demesmay et al. (74) separated the 6 principal As species using a gradient of pH 6.5 and 7.95 (Figure 2). The chromatographic separation shows that Aschol (cationic), Asbet (zwitterion), and As(III) (neutral) are the first

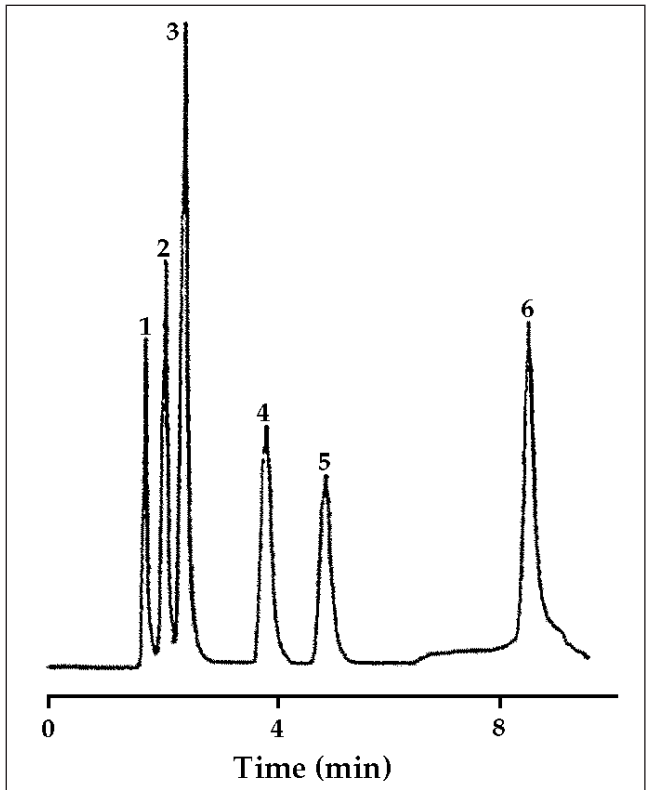


Figure 2. Anion-exchange chromatography of 6 arsenic compounds in step-gradient elution mode by HPLC–ICP-MS with an ion-echange ploymer-based column. Peaks: 1, Aschol; 2, Asbet; 3, As(III); 4, DMA; 5, MMA; 6, As(V).

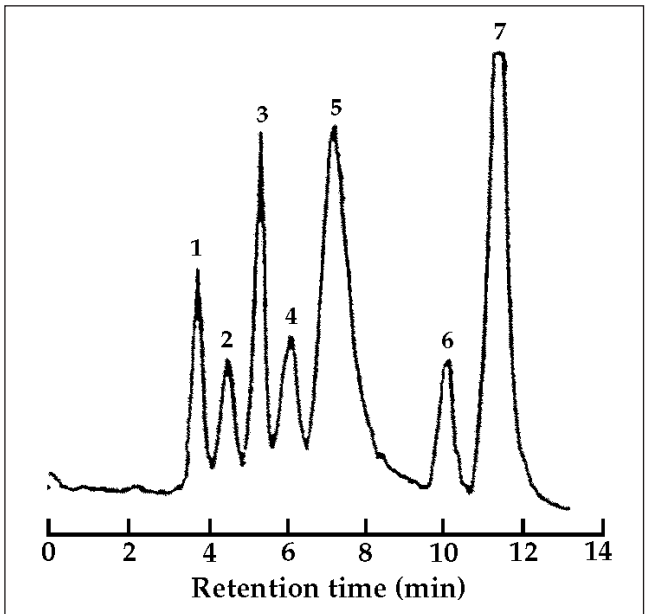


Figure 3. Anion- and cation-pair chromatography of 7 arsenicals obtained on a reversed-phase C_{18} column coupled with HPLC–MW-HG-AFS. Peaks: 1, As(V); 2, As(III); 3, Asbet; 4, MMA; 5, DMA; 6, Aschol; 7, TMA.

eluted species; their separation is not attributed to an ion-exchange phenomenon but rather to the introduction of an organic modifier (acetonitrile) in the mobile phase, allowing the separation of Asbet from Aschol. At neutral pH, in the ion-pairing mode, un-ionized As(III) and Asbet (zwitterion) cannot give ion-pairs and are eluted together in the void volume, whereas Aschol is retained on the C_{18} column by hydrophobic interactions (75,76). Nevertheless, Mingsheng and Norris (40) succeeded in separating 7 arsenicals on a C_{18} phase by using elevated column temperatures (70°C) and mixed (anionic and cationic) ion-pair reagents (hexanesulfonate and tetraethylammonium hydroxide) (Figure 3). Multiple modes of separation mechanisms are probably responsible for the excellent separation: ion-pairing, ion-exchange, hydrophobic interactions with the C_{18} moiety and silanol groups. The entire separation of the totality of the As species necessitate operation at alkaline pH values (> 9). This is possible with a polymeric column or a stabilized bonded C_{18} silica column (STM OD-5, 100 µm). Nevertheless, the use of alkaline mobile phases does not allow the separation of Aschol (cationic) and Asbet (zwitterion), which elute in the solvent front. Terasahde et al. (77) succeeded in the separation of the 6 As solutes by on-line chromatography on an anionic column followed by a cationic column (Figure 4). The polymeric anion column was able to function over a pH range of 1–12. A gradient pH program was used. A diluted carbonate solution of pH 9.3 first allowed the retention of As(III), As(V), DMA, MMA, and Aschol on the anionic column while Asbet eluted in the void volume; then, by increasing the carbonate concentration and slightly decreasing the pH with nitric acid, the species were eluted from the column. However, Aschol coeluted with DMA.

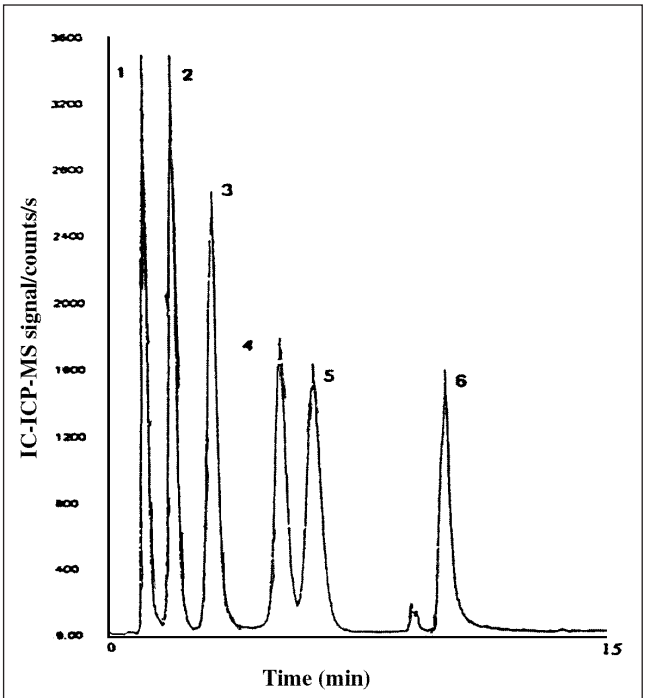


Figure 4. Anion-exchange and cation-exchange chromatography of 6 arsenic species using HPLC–ICP-MS, a cation guard column, and an anion column in step gradient elution mode. Peaks: 1, Asbet; 2, DMA; 3, As(III); 4, MMA; 5, As(V); 6, Aschol.

Because Aschol has a cationic character, it was strongly retained on the cation guard column coupled online with the anion column. Aschol was eluted from the cation guard column with nitric acid. With the ion-pair mode, all compounds were separated at pH 9 on a unique column (33,42) (Figure 5). The separation between Aschol and Asbet can be increased by the addition of a counter-cation in the mobile phase (29,40).

The time of analysis can be reduced by using a gradient (78), but the isocratic mode has the advantage of keeping the chromatographic system in a permanent equilibrium that is more convenient for routine use. Indeed, this technique has a fast kinetic of exchange and good efficacy (79).

Ion-pairing is based on the distribution of ion-pairs between the aqueous mobile phase and the hydrophobe stationnary phase: the nonpolar stationary phase can be a C_{18} bonded silica (64,75,80,81) or a polymeric resin (42,53,76,82). The counter-ion bears several alkyl chains. For the separation of the anionic species, the counter-ion is a quaternary ammonium. The more frequently used reagents are tetrabutylammonium phosphate (TBAP) (33,42,83) and tetrabutylammonium hydroxide (TBAH) (75,76,80). Tetraethylammonium hydroxide (TEAH) (40) and tetraheptylammonium nitrate (THAN) (84) have also been used. Larsen and Hansen (85) have used ammoniums with long alkyl chains (C_8–C_{12}) and the pyridinium ion.

For the separation of cationic species, the counter-ion is a long alkyl chain sulfonate, such as hexanesulfonate (40) or butanesulfonate (85). The pH of the mobile phase varies between 4 (40) and 9 (33,42). The elution is made in the isocratic or gradient mode, sometimes at elevated temperatures (70°C) to reduce the elution time (40). The elution is effected between 6 and 13 min (Table III). The separations are applied to urine (10,26,72), serum (10,26,55), or tissue extracts of marine organisms (29,42,80,86) (Table III). Ion-exchange separations are especially concerned with ionized or ionizable species, although non-ionic species can also be separated. Separations of all As species present in biological media necessitate the use of 2 different columns, anionic and cationic (77).

The increase in the column temperature increases the efficiency of separation by increasing the kinetic of exchange, which is very slow compared to ion-pair separations (65). Several types

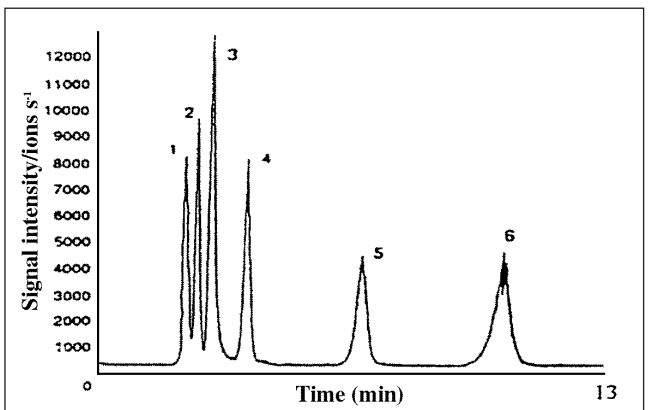


Figure 5. Anion-pair chromatography of 6 arsenic species using HPLC–ICP-MS and a reversed-phase C_{18} column. Peaks: 1, Aschol; 2, Asbet; 3, As(III); 4, DMA; 5, MMA; 6, As(V).

Table III. Arsenic Speciation: Technical Characteristics of the Methods

Species (in order of elution)	Sample	Technique	Chromatographic conditions	Time of separation	Detection limit	Sample amount	Reference
Asbet, As(III), MMA, DMA, As(V)	seaweed	HPLC–ICP-AES	column, anion-exchange Nucleosil-$N(CH_3)_3$-10 Nagel; mobile phase, phosphate buffer (25mM); pH 7.0	15 min	30 ng As(III), 19 ng MMA, 41 ng DMA, 30 ng As(V)	—	73
As(III), DMA, MMA, As(V)	fish tissue	HPLC–HG-ICP-AES	column, 10-µm anion-exchange resin Nucleosil and PRPX-100 (250 × 4.1-mm i.d.); mobile phase, phosphate buffer; gradient, 100 and 50mM; pH 5.75	8 min	3.5 µg/L As(III), 21.3 µg/L DMA, 3.8 µg/L MMA, 9.2 µg/L As(V)	100 µL	89
Aschol, Asbet, As(III), DMA, MMA, As(V)	mussel tissue, dogfish muscle cod-muscle	HPLC–UV-HG-ICP-AES	column, 10-µm anion-exchange resin Hamilton PRPX-100 (250 × 4.1-mm i.d.); mobile phase, phosphate buffer; gradient, 5 and 35mM; pH 6.0	15 min	0.23 µg/g Aschol, 0.28 µg/g Asbet, 0.30 µg/g DMA, 0.31 µg/g MMA, 0.30 µg/g As(V)	20 µL	44
As(III), DMA, MMA, As(V)	water	HPLC–HG-QFAAS	column, 10-µm anion-exchange resin PRPX-100 (250 × 4.1-mm i.d.); mobile phase, phosphate buffer; gradient, 20 and 100mM; pH 6.0	11 min	1.3 µg/l As (III), 4.6 µg/1 DMA, 3.8 µg/1 MMA, 4.8 µg/l As(V)	100 µL	90
As(III), DMA, MMA, As(V)	seafood (shellfish, sardine, tuna)	HPLC–HG-QFAAS	column, 10-µm anion-exchange resin PRPX-100 (250 × 4.1-mm i.d.); mobile phase, phosphate buffer; gradient, 20 and 100mM; 28°C column temperature; pH 5.75	10 min	3 ng/g DMA, 2 ng/g MMA	100 µL	92
Asbet+As(III), DMA, MMA, Aschol, As(V), pAPA	river water	HPLC–HG-GFAAS	column, anion-exchange Elite AS-3 (250 × 4.6-mm i.d.); mobile phase, 5mM ammonium phosphate buffer; pH 5.8	15 min	1.9 µg/L Asbet, 1.6 µg/L As(III), 1.9 µg/L DMA, 1.6 µg/L MMA, 1.6 µg/L As(V), 1.9 µg/L Aschol, 1.6 µg/L pAPA	100 µL	94
Asbet, DMA, As(III), MMA, As(V), Aschol	river water	HPLC–ICP-MS	cation column coupled online with anion column; mobile phase, gradient $NaHCO_3$–Na_2CO3 (0.3, 2.5, and 10mM) and HNO3 (4, 6, and 40mM)	12 min	0.10 µg/L Asbet, 0.08 µg/L DMA, 0.10 µg/L As(III), 0.19 µg/L MMA, 0.30 µg/L ASM, 0.24 µg/L Aschol	100 µL	77
Aschol, Asbet, As(III), DMA, MMA, AS(V)	spring water	HPLC–ICP-MS	column, 10-µm PRP-1 Hamilton (250 × 4.6-mm i.d.); mobile phase, 4mM phosphate buffer–0.5 mM TBAP; pH 9.0	13 min	1.4 µg/L Asbet, 0.9 µg/L As(III), 1.5 µg/L DMA, 3.0 µg/L MMA, 2.6 µg/L As(V), 1.4 µg/L Aschol	50 µL	33
As(III), Asbet, MMA, DMA, As(V)	urine	HPLC–ICP-MS	column, 5-µm anion-exchange resin Adsorbsphere-NH2 (250 × 4.6-mm i.d.); mobile phase, 15mM $NH_4H_2P0_4$–1.5mM CH_3COONH_4; pH 5.7	22 min	—	100 µL	54
(As(III)+As(V)+MMA), DMA, Asbet, Aschol	dog-fish	HPLC–ICP-MS	column, 5-µm cation-pairing C_{18} Pierce (300 × 4.6-mm i.d.); mobile phase, 0.01M dodecylphosphate desodium–5% methanol–2.5% acetic acid	6 min	1.5 µg/L Asbet	200 µL	29
Asbet, As(III), As(V), Arsenosugar and dimethyl arsinoylethanol	urine	HPLC–ICP-MS	column, Intersil and Asaphipak ion-exchange; mobile phase, 1.25mM tetramethylammonium, 5% methanol; pH 6.8	12 min	20 ng/L As(III),	100 µL	91

Table III continued

Species (in order of elution)	Sample	Technique	Chromatographic conditions	Time of separation	Detection limit	Sample amount	Reference
As(III), DMA, MMA, As(V)	water	HPLC–HG-ICP-MS	column, 5-µm C_{18} Vydac 201 (250 × 4.6-mm i.d.); mobile phase, 2mM TBAP–2mM ammonium acetate, 2% methanol; pH 5.9	10 min	150 ng/L As(V) 0.011 µg/L As(III), 0.018 µg/L DMA, 0.029 µg/L MMA, 0.051 µg/L ASM	200 µL	60
DMA, As(III), MMA, As(V)	urine	HPLC–ICP-MS	micellar liquid chromatography column Hamilton PRP-1 (150 × 4.1-mm i.d.); mobile phase, 0.05M cetyltrimethylammonium bromide–10% propanol, 0.02M borate buffer; column temperature, 40°C; pH 10.2	15 min	0.9 µg/L DMA, 3 µg/L As(III), 3 µg/L MMA, 3 µg/L As(V)	100 µL	72
As(III), DMA, MMA, As(V), para-arsanilic acid (pASA)	arsenic animal feed addifives	µHPLC–ICP-MS	microbore column Spherisorb 3-µm C_{18} (150 × 1-mm i.d.); mobile phase, 1mM TBAH–0.5% methanol; pH 5.28; flow rate, 40 µL/min	10 min	0.1 µg/L As(III), 0.1 µg/L As(V), 0.1 µg/L pASA	60 µL	112
Ion-pairing (1): As(III), DMA, MMA, As(V); Cation-exchange (2): MMA, DMA, Asbet, Aschol	Serum, dialysate	HPLC–UV-HG-QFAAS	ion-pairing column, C_{18} RP Biorad (250 × 4.6-mm i.d.); mobile phase, 10Mm TBAP–20mM phosphate buffer; pH 6.0; cation exchange column, cation exchange resin Dionex Ionpac CS10 (250 × 4-mm i.d.); mobile phase, 100mM HCl–50mM NaH_2P04	10 min	1 µg/L DMA, 1.3 µg/L MMA, 1.5 µg/L Asbet, 1.4 µg/L Aschol	100 µL	26,55
As(V), As(III), Asbet, MMA, DMA, Aschol, TMA	urine, seaweed	HPLC–MW-HG-AFS	column, 5-µm C_{18} ODS 100 (300 × 3.9-mm i.d.); mobile phase, 10mM hexanesulfonate–1mM TEAH and 0.5% methanol; column temperature, 70°C; pH 4.0	13 min	—	20 µL	40
Aschol, Asbet, As(III), DMA, MMA, As(V)	fish, sediment	HPLC–ICP-MS	column, 10-µm anion-exchange resin Hamilton PRPX-100 (250 × 4.1 mm i.d.); mobile phase gradient A, 10mM $(NH4)H_2PO4$– $(NH4)_2HPO4$ and 2% CH_3CN; pH 6.5; mobile phase gradient B, 100Mm $(NH4)_2HPO4$; pH 7.95	10 min	0.5 µg/L Aschol, 0.5 µg/L Asbet, 0.5 µg/L As(III), 1 µg/L DMA, 1 µg/L MMA, 1.5 µg/L As(V)	20 µL	74
As(III), DMA, MMA, As(V)	urine, wine, club-soda	HPLC–ICP-MS	column, Wescan anion-exchange (250 × 4.1-mm i.d.); mobile phase, 50mM carbonate buffer; pH 7.5	12 min	4.9 µg/L As(III), 1.2 µg/L DMA, 3.6 µg/L MMA, 6µg/L As(V)	100 µL	57
As(III), DMA, MMA, As(V)	urine	HPLC–ICP-MS	column, 5-µm anion-exchange resin Adsorbosphere-NH_2 (250 × 4.6-mm i.d.); mobile phase, 30% methanol and 15mM $(NH4)_2HPO4$–1.5mM CH_3COONH_4; pH 5.75	10 min	1.46 µg/L As(III), 0.76 µg/L DMA, 0.72 µg/L MMA, 1.82 µg/L As(V)	50 µL	56
Aschol, Asbet, As(III), DMA, MMA, As(V)	fish, mussel	HPLC–ICP-MS	column, 10-µm Hamilton PRP-1 (250 × 4.1-mm i.d.), mobile phase, 0.5mM PTBA–4mM phosphate buffer; pH 9	13 min	9 µg/L Aschol, 6 µg/L As, 6 µg/L Asbet, (III), 10 µg/L DMA, 22 µg/L MMA, 25 µg/L As(V)	20 µL	42
Asbet, As(III), DMA, MMA, As(V)	dogfish	HPLC–ICP-MS	anion-pairing column, 10-µm RP-1 (150 × 4.1-mm i.d.); mobile phase, 0.5mM TBAH and 5% methanol; pH 7.0; anion-exchange column, PRPX-100 (Hamilton); mobile phase, phosphate buffer 8 mM; pH 7.0; cation-pairing column PRP-1 (Hamilton); mobile phase, 5% methanol–2.5% acetic acid and 50mM sodium dodecylsulphate; pH 2.5	9 min	1 µg/L As(III), 5 µg/L Asbet	200 µL	80

of stationary phases have been used: C_{18} bonded silicas (74,75,83,87) or polymeric phases (44,85,88,89) bearing exchange sites of anionic type (quaternary ammoniums) (44, 59, 74) or cationic type (sulfonate) (77,80).

Compounds are eluted either by a phosphate (74,89,90), carbonate (57), or acetate (54) buffer. The pH values range between 2.5 (58,80) and 7.0 (73,91) for the classical bonded C_{18} columns and between 9 (33,42) and 10 (72,74) for columns resistant to alkaline pH. The addition of an organic modifier leads to a lowering of the hydrophobic interaction with improvement of the chromatographic separation (73,74,76,87). The duration of the analysis varies from 10 to more than 20 min (Table III).

Separations by ion-exchange have been applied to As speciation in marine foods (44,53,92), serum (26,55), urine (56,57, 59,93), dialysis liquids, and water (33,60,94–96).

Cationic ion-exchange has been applied to several types of natural samples for the speciation of arsenical species in marine foods (80,97), urine (26,55,98), and blood serum (26,55) (Table III).

A third mode of separation is micellar liquid chromatography (MLC). MLC refers to the type of chromatography that uses surfactants in aqueous solutions well above their critical micelle concentration as alternative mobile phases for reversed-phase LC (RPLC). In addition to the number of compounds with a wide range of polarities amenable to separations by RPLC, MLC extends the analyte candidates to almost all hydrophobic and many hydrophilic compounds, provided they can partition from the micelles (72,79). MLC offers other advantages over RPLC, such as the simultaneous separation of both ionic and non ionic compounds, faster analysis times, and improved detection sensitivity and selectivity (72). MLC stems from its unique 3-way equilibrium mechanism in which micelles act as a pseudo-phase in addition to the mobile and stationary phases. The presence of micelles in the mobile phase improves the interactions of solutes with the stationary phase (79).The amount of organic solvent in the mobile phase is then considerably decreased in this method; that could be an advantage for ICP-MS determination. This method also presents better separation power when the temperature of the column is increased.

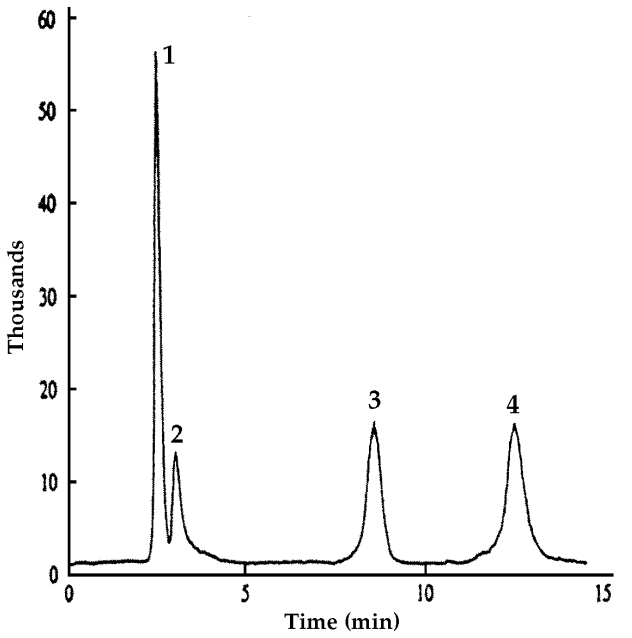


Figure 6. Micellar liquid chromatography of 4 arsenic species using HPLC–ICP-MS and a reversed-phase C_{18} column. Peaks: 1, DMA; 2, As(III); 3, MMA; 4, As(V).

MLC was also studied by Ding et al. (72) for the separation of 4 arsenicals (As[III], As[V], DMA, MMA) as a function of pH, concentration of surfactant, and percent of organic solvent in the mobile phase.The speciation of these compounds in urine (Figure 6) shows an incomplete separation of DMA and As(III), which are eluted in the solvent front. Indeed, the method gives a fairly good separation of chlorides from As species, which is an advantage in ICP-MS. The main focus of this work (72) was to investigate the use of MLC for direct injection of "dirty samples" and a better compatibility with ICP-MS. With conventional hydroorganic mobile phases, "dirty samples" such as body fluids usually have to be deproteinized before chromatography to prevent protein precipitation in the column. However, the unique micelle aggregates in MLC should dissolve proteins in the sample and cause them to elute with the void volume without plugging the column. In MLC, the possibility of direct sample introduction should greatly simplify sample treatment and improve accuracy. In addition, MLC should give better compatibility with ICP-MS than RPLC. This is because MLC uses no or low concentrations of organic solvents, whereas the high organic content in RPLC usually leads to plasma instability, increased background, and carbon deposition on the sampling cone of the ICP.

Detection of arsenic species

Generally, the detection systems used are those compatible with liquid chromatography. Classical detectors such as UV and electrochemistry (amperometry and coulometry) lack sensitivity and selectivity, and their use is therefore limited, except for As speciation in water (99) or in standard solutions during the study of arsenical separations (75,76,100).

For speciation in biological media, the main detection systems used are high frequency inductive plasma atomic emission spectrometry (ICP-AES), QF-AAS, ICP-MS. Atomic fluorescence spectrometry is less frequently used. All detectors are directly coupled to the LC system.

ICP-AES detection

ICP-AES detection is the most frequently used method. It has the advantage of ease of use because of the similarity of flows at the end of the chromatographic column and at the entrance in the detector. The interface between both systems is easy to carry out with a simple connection made of inert materials (PEEK or polytrifluoro ethylene) to link the end of the column with the entry of the nebulizer (78,83). Nevertheless, the ICP-AES system allows only some little percentage (less than 5%) of organic solvents, because they destabilize the ICP plasma and lead to elevated background levels (78,101). Therefore, this detector is well adapted to the speciation of As by LC (44,73,89), either by ion-exchange, ion-pairing, or micellar chromatography. This technique has the advantage of sensitivity, with detection limits generally above the levels commonly found in biological samples (44,73,78,86), as well as the capacity of multielement analysis with a high resolution power towards various spectral emission

lines (83,102). Plasmas are either generated by direct current (DCP) or inductively coupled plasma (ICP) (102).

The chromatographic liquid flows are compatible with those (1 mL/min) generally used in ICP-AES. This coupling method has been developed for As speciation with ion-exchange (44,83,89,103) or ion-pair chromatography (104). The main difficulty is generally the presence of organic solvents used in reversed-phase separations. So, a very accurate and careful setting of the various parameters of the equipment is necessary to obtain a stable plasma. This coupling method is especially convenient for As concentrations in the range of several micrograms per gram in solid samples and micrograms per liter in liquids. To increase the detection limits, some authors have proposed modifications in the sampling system for introduction into the spectrometer. For example, La Freniere et al. (83) used a microcentric nebulizer placed inside the torch (direct injection nebulizer, DIN) instead of the conventional pneumatic nebulizer. The liquid sample was introduced directly into the plasma through a capillary, where it produced a mist of fine droplets. With this apparatus, nearly 100% yields of nebulization are reached, and mobile phases with 100% organic solvent can be used; thinner chromatographic peaks are therefore obtained because of the reduction of extra-column effects (reduction of the dead volume, suppression of the nebulization chamber) (105). Absolute detection limits are reported to be one order of magnitude better when compared with conventional pneumatic nebulizers (102,105). This improvement in absolute detection limits can be explained by the complete nebulization of the sample into the plasma; no loss of analyte occurs. The absence of a spray chamber and a very low dead volume make it a good candidate for coupling with microcolumns (106). It has been found that the plasma has a greater tolerance to solvents from the DIN. This can possibly be attributed to the high linear velocity with which the aerosol passes through the axial channel of the plasma. Nevertheless, this type of nebulizer can be used only with liquid flows in the range of 100 to 200 µL/min (105,106). Therefore, it necessitates separation on a microcolumn (microbore) (106) or a division of the chromatographic effluent to obtain a compatibility of flows (105).

The sensitivity of ICP-AES can also be greatly improved with the use of HG techniques because of a more efficient sample introduction and matrix removal. Hydride generation consists of transforming the organometallic molecules in volatile species by sodium tetrahydroborate reduction. Compounds thus formed are then separated from the liquid phase and conducted by an inert gas flow inside the detection cell. This reaction leads to lower detection limits first by separating solutes from the matrix and secondly by increasing the efficiency of introduction into the detector (44,89). Several As species can be determined by hydride generation if selective reduction is employed by carefully controlling the pH (89,94,96). However, a number of important organoarsenicals (Asbet, Aschol) are "nonreducible" (i.e., they do not form volatile hydrides, thus they are not detected by this approach) (36,89). Therefore, decomposition of these species in a form that is compatible with the HG generation technique is required prior to analysis. Decomposition can be performed by a number of methods including dry-ashing (41–43), wet digestion (44–46), and photo-oxidation (104,107). With these methods, Asbet and Aschol (as well as MMA and DMA) are converted into As(V). Nevertheless, these techniques are not adequate for online measurement because of a very long reaction time for Asbet (several hours). In order to improve these methods, UV degradation of the As species in the presence of persulfate has been used for the determination of Asbet, DMA, MMA, and As(V) by HPLC–HG-ICP-AES (44,89) and other systems (26,55). Alberti et al. (44) studied the photooxidation (shape of the photoreactor, irradiation time, and power of the lamp were optimized) of Asbet and Aschol in presence of persulphate at several concentrations; with these conditions, the conversion of arsenicals into As(V) is total, allowing their quantitation by HPLC–HG-ICP-AES. However, this technique is not convenient because, following decomposition, the HG response of different arsenic species is not uniform (65). The detection limits are in the nanogram-per-gram range, but this coupling method has few utilizations because of its high cost in comparison with HPLC–HG-QF-AAS, in which detection limits are in the same range (Table III).

Detection by AAS

GFAAS is often preferred to flame AAS, because sensitivity is clearly greater by a factor of 10 or 100 (108). Indeed, the coupling of a separating technique such as HPLC with a sequential detector is not easy. Two types of coupling have been developed. The first method, online coupling, consist of utilization of the automatic sampler of the spectrometer as a fraction collector. Fractions are then injected in the furnace and analyzed. The resolution is good, but the time of analysis is elevated (108). In the second method, indirect coupling, the chromatographic effluent fractions are collected and periodically analyzed. This technique is only applicable if the chromatographic peaks are wide enough, because 30 to 60 s are necessary for every determination (108). The sensitivity of this coupling is mainly dependent on the characteristics of the oven and the chromatographic system used. The detection limits are in the range of a few nanograms (90,92,94) (Table III).

Presently, atomic absorption detection is more frequently carried out with QF spectrometers (92,94). The entire system is an online including: the HPLC system, a UV or microwave (MW) digestion system, HG, and the detector (QF-AAS) (HPLC–UV-HG-QF-AAS or HPLC–MW-HG-QF-AAS) (26,55,61,65). The hydride generating techniques are identical to those previously described. The benefit of prereduction of L-cysteine prior to analysis using hydride generation techniques are well documented in the literature (65). Following prereduction, all As species are present in the same oxidation state (As[III]), thus giving the same response for HG. Sensitivity is increased, because the formation of hydrides from As(III) is more efficient than from As(V). In addition, less interference from transition metals such as Cu, Co, Ni, and Pd is generally experienced (109). Lamble and Hill (65) used an online system capable of both speciation and total As determination in biological samples using microwave digestion and HG-AAS. In order to achieve the latter, an L-cysteine prereduction was combined with the initial potassium persulphate microwave digestion. The system can also be adapted to determine the total reducible As species in the sample. The prereduction step allows increasing sensitivity, because hydride generation from trivalent arsenicals has a relatively high yield. The detection limits of this technique are in the nanogram-per-gram range (92).

Detection by ICP-MS

ICP-MS is widely known as a powerful technique for trace element analysis and is being increasingly used as a detector for chromatographic determinations. The benefits of coupling plasma MS detection with chromatographic separation include element specificity, real-time chromatograms, the ability to separate interferences from peaks of interest, multielement capability, and low levels of detection (sub-nanogram for most elements) (33,42,110). These features of ICP-MS are valuable assets in the speciation of trace elements, where pretreatment should minimized. Sample pretreatment and preconcentration can lead to changes in the relative concentration of individual species. ICP-MS is 100- to 1000-fold more sensitive than ICP-AES (110).The greater sensitivity of plasma source MS detection can often reduce or eliminate the need for a time-consuming preconcentration and/or derivatization step.

ICP-MS is particularly interesting because of its ability to measure isotope ratios on eluting peaks with good precision (< 2%) and accuracy (1%). Isotope dilution would decrease analysis time in comparison with a calibration curve method and would help compensate for matrix effects (111).

One problem associated with the determination of As using ICP-MS is the polyatomic interference at *m/z* 75. The chloride ion present in biological samples combines with the plasma gas to form $^{40}ArCl^+$ (*m/z* = 75), which is detected together with As species (*m/z* = 75), thus yielding inaccurate results. Chloride could be separated chromatographically from As species present in samples using ion exchange (57,58), ion pair (112), or MLC (72), but the resolution is poor, and the chloride peak is intense, because chloride concentrations in urine are approximately 105 times more concentrated than any of the As species present (57). Hutton (113) has shown that a 5% chlorhydric acid solution gives a peak equivalent to a solution containing 6 µg/L of As. In another study, Heitkemper et al. (56) observed that the abundance of the $ArCl^+$ ion is not only due to chloride concentration but also to factors in relation to the equipment such as the plasma sampling position and other concomitant elements. From a practical point of view, several approaches have been proposed for the elimination of this interference (see Sample preparation). The chromatographic separation is the best method but is difficult to develop without completely changing the chromatographic system (53,57). Another method being investigated involves the use of a helium microwave induced plasma as an alternative ion source. By eliminating or reducing the argon from the excitation source, $ArCl^+$ formation should be eliminated or significantly reduced (56,59). Another problem is the necessity of low concentrations of organic solvent (< 5%) in the mobile phases. The introduction of organic solvents can destabilize the ICP and lead to elevated background levels associated with carbon deposition at the MS sampling cone (57,72,111).

Additionally, alternative ionization sources such as the He–Ar mixed-gas ICP may be used to enhance the sensitivity of plasma MS (57,58). The He–Ar mixed gas combines the familiar features of an ICP with the benefits of the plasmas. Plasmas containing He are generally thought to be more energetic than their Ar counterparts because of the higher ionization energy and metastable state energy of He. Higher plasma energies are required to achieve better sensitivity with higher ionization potential elements such as the halogens As and Se. The addition of He to Ar plasmas has been shown to increase the sensitivity for elements with higher ionization energies while maintaining high sensitivity for the other elements (57).

The detection limits can be further lowered by the addition of a postcolumn reaction such as hydride generation (60) or by changing the nebulizer type to direct injection nebulizers (DIN) (60), high-efficiency nebulizers (HEN) (106), or microcentric nebulizers (MCN) (105). They lead to the possibility of coupling HPLC with microbore columns (µHPLC–ICP-MS) (112,114). HEN is a low-flow nebulizer with a small capillary and a spray chamber. Compared with conventional pneumatic nebulizers, HEN operates more efficiently at very low solution uptake rates. A droplet-size distribution was found to be narrower than that from a DIN operating at flow rates of less than 100 µL/min. Possible drawbacks of the HEN, as with all low-flow nebulizers, is the poor tolerance in nebulizing highly concentrated solutions. A µHPLC–ICP-MS coupling was used for the speciation of 5 As species by Pergantis et al. (112).

An MCN is a low-flow concentric pneumatic nebulizer designed to analyze low volume samples, a high concentration of mineral acids, and samples containing high levels of dissolved solids. The MCN itself includes a spray chamber endcap adapter that allows the nebulizer to be connected to standard spray chambers on most ICP-MS and ICP-AES instruments (114). This nebulizer was used for speciation of arsenic in urine (72) and in water (77) by HPLC–ICP-MS. The HPLC–ICP-MS technique is very effective for the speciation of trace element toxics such as As in various natural samples. The detection limits are in the range of a few nanograms per liter. This technique was used with ion exchange (56,57,74,80), ion pairs (33,42,80,112), and liquid micellar chromatography (72). It was applied to As speciation in various biological samples (Table III).

Conclusion

Arsenic speciation in biological samples (human or dietary) is an important analytical problem because of the complex matrix and low level of analyte concentrations. Proteins and salts are the main matrix interferents either in the HPLC step or during the detection. Many sample pretreatment methods have been proposed, but each gives different results between samples and must be adapted to each sample. The difficulty of the chromatographic separation of As species originates in their very close physicochemical properties. It is therefore necessary to use special alkaline-stable columns or monitor the online coupling of 2 different columns. Ion-pair separations at pH 9.0 are of particular interest.

The ICP-MS detector is the most sensitive and specific, but also has an increased cost. Because of the sensitivity, the preconcentration step can be omitted. Indeed, the chloride ion gives a spectral interference that could be eliminated by the use of a helium plasma instead of argon. ICP-AES and AAS are sensitive enough methods for biological media, and the sensitivity can be further enhanced by HG.

References

1. D.A. Grantham and J.F. Jones. Arsenic contamination of water wells in Nova Scotia. *J. Am. Water Works Assoc.* **69:** 653–61 (1977).
2. N. Mizuta, M. Mizuta, F. Ito, and T. Ito. An outbreak of acute arsenic poisoning caused by arsenic contaminated soy sauce. *Bull. Yamagushi Med. School.* **4:** 131–37 (1956).
3. G. Pershagen. The epidemiology of human arsenic exposure. In *Biological and Environmental Effects of Arsenic*, B.A. Fowler, Ed. *Top. Environ. Health* **6:** 199–217 (1983).
4. N. Yamashita, M. Doi, and M. Nishio. Recent observations of Kyoto children poisoned by arsenic tainted Morinaga Dry Milk. *Jpn. J. Hygiene.* **27:** 364–72 (1972).
5. M. Börzsönyi, A. Bereczky, P. Rudnai, M. Csanady, and A. Horvath. Epidemiological studies on human subjects exposed to arsenic in drinking water in Southeast Hungary. *Arch. Toxicol.* **66:** 77–78 (1992).
6. J.R. Goldsmith, M. Deane, J. Thom, and G. Gentry. Evaluation of health implications of elevated arsenic in well water. *Water Res.* **6:** 1133–41 (1972).
7. J.M. Harrington, J.P. Middaugh, D.L. Morse, and J. Housworth. A survey of a population exposed to high concentrations of arsenic in well water in fairbanks Alaska. *Am. J. Epidimiol.* **108:** 377–86 (1978).
8. M.A. Lovell and J.G. Farmer. Arsenic speciation in urine from human intoxicated by inorganic arsenic compounds. *Human Toxicol.* **4:** 203–214 (1985).
9. M.W. Arbouine and H.K. Wilson. The effect of seafood consumption on the assessment of occupational exposure to arsenic by urinary arsenic speciation measurements. *J. Trace Elem. Electrolytes Health Dis.* **6:** 153–60 (1992).
10. M. Buratti, G. Calzaferri, G. Caravelli, A. Colombi, M. Maroni, and V. Foa. Significance of arsenic metabolic forms in urine. Part I: chemical speciation. *Intern. J. Environ. Anal. Chem.* **17:** 25–34 (1984).
11. W. Goessler, W. Maher, K.J. Irgolic, D. Kuehnelt, C. Schlagenhaufen, and T. Kaise. Arsenic compounds in marine food chain. *Fres. J. Anal. Chem.* **359:** 434–37 (1997).
12. J.O. Nriagu and M.S. Simmons. *Food Contamination from Environmental Sources.* John Wiley and Sons, New York, NY, 1990, pp 121–39.
13. A. Franzblau and R. Lilis. Acute arsenic intoxication from environmental arsenic exposure. *Arch. Environ. Health* **44:** 385–90 (1989).
14. W. Takahashi, K. Pfenninger, and L. Wong. Urinary arsenic, chromium, and copper levels in workers exposed to arsenic-based wood preservatives. *Arch. Environ. Health* **38:** 209–14 (1983).
15. J.T. Hindmarsh and R.F. McCurdy. Clinical and environmental aspects of arsenic toxicity. *Crit. Rev. Clin. Lab. Sci.* **23:** 315–47 (1986).
16. *Clinical Toxicology,* C.J. Poison and R.N. Tattersal, Eds. Pitman, London, England, 1969, pp 181–84.
17. R.L. Tatken and R.J. Lewis. *Registry of Toxic Effects Chemical Substances.* U.S. Department of Health and Human Services, Cincinnati, OH, 1983.
18. L.D. Fitzgerald. Arsenic sources, productions and applications in the 1980's. In *Arsenic, Industrial, Biomedical, and Environmental Perspectives*, W.H. Lerdere and R.J. Frernstein, Eds. Van Nostrand Reinold Co., New York, NY, 1983, pp 3–8.
19. R. Bauer. Arsenic: glass industry requirements. In *Arsenic, Industrial, Biomedical, and Environmental Perspectives*, W.H. Lerdere and R.J. Frernstein, Eds. Van Nostrand Reinold Co., New York, NY, 1983, pp 45–55.
20. T.A. Haupert, J.H. Wiersma, and J.M. Goldring. Health effects of ingesting arsenic-contaminated groundwaste. *Wis. Med. J.* **95:** 100–104 (1996).
21. Ferreccio, C. Gonzalez, J. Safari, and C. Noder. Broncho-pulmonary cancer in workers exposed to arsenic: a case central study. *Fev. Med. Chil.* **124:** 119–23 (1996).
22. R.C. Hopenhayn, M.L. Biggs, A. Fuchs, R. Bergoglio, E.E. Telo, H. Nicolli, and A.H. Smith. Bladder cancer mortality associated with arsenic in drinking water in argentina. *Epidemiology* **7:** 117–24 (1996).
23. W. Morton, G. Staff, J. Pohl, and S. Stoner. Skin cancer and water arsenic in Lane County Oregon. *Cancer* **37:** 2523–31 (1976).
24. N. Yamato. Concentration and chemical species of arsenic in human urine and hair. *Bull. Environ. Contamin. Toxicol.* **40:** 633–40 (1988).
25. X. Zhang, R. Cornelis, J. DeKimpe, L. Mees, and N. Lameire. Speciation of arsenic in serum, urine, and dialysate of patients on continuous ambulatory peritoneal dialysis. *Clin. Chem.* **43:** 406–408 (1997).
26. X. Zhang, R. Cornelis, J. DeKimpe, L. Mees, V. Vanderbiesen, A. DeCubber, and R. Vanholder. Accumulation of arsenic species in serum of patients with chronic renal disease. *Clin. Chem.* **42:** 1231–37 (1996).
27. P. Michel. *Arsenic in marine medium, Biochemistry and Ecotoxicology.* Repères. Océan N°4. Ed. IFREMER Paris. (1993).
28. M. Navarro, H. Lopez, M.C. Lopez, and M. Sanchez. Determination of arsenic in fish by hydride generation atomic absorption spectrometry. *J. Anal. Toxicol.* **16:** 169-71 (1992).
29. D. Beauchemin, M.E. Bednas, S.S. Berman, J.W. Mclaren, K.W.M. Siu, and R.E. Sturgeon. Identification and quantification of arsenic species in dogfish muscle reference materiel for trace elements. *Anal. Chem.* **60:** 2209–2212 (1988).
30. W.T. Piver. Mobilization of arsenic by natural and industrial process. In *Biological and Environmental Effects of Arsenic,* B.A. Fowler, Ed. *Top. Environ. Health* **6:** 1–7 (1983).
31. R.S. Braman. Environmental reaction analysis methods. In *Biological and Environmental Effects of Arsenic*, B.A. Fowler, Ed. *Top. Environ. Health* **6:** 141–46 (1983).
32. J.K. Nag, V.B. Balaram, R. Rubio, J. Alberti, and A.K. Das. Inorganic arsenic species in groundwater: a case study fom Perbasthali (India). *J. Trace Elem. Med. Biol.* **10:** 20–24 (1996).
33. P. Thomas and K. Sniatecki. Determination of trace amounts of arsenic species in natural waters by high performance liquid chromatography inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **10:** 615–18 (1995).
34. R.A. Dehove. Regulations of food products. *Commerce. Ed.* Paris **10:** 994–95 (1981).
35. *U.S. EPA Quality Criteria of Water*, report EPA 440/5-86-001. Office for Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, DC, 1986.
36. N. Furuta and T. Shinfuji. Determination of different oxidation states of arsenic and selenium by inductively coupled plasma–atomic emission spectrometry with ion chromatography. *Fres. J. Anal. Chem.* **355:** 457–60 (1996).
37. *Environmental Health Criteria. 18. Arsenic WHO.* World Health Organization, Geneva, 1981.
38. J.H. Farmer and L.R. Johnson. Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. *Brit. J. Ind. Med.* **47:** 342–48 (1990).
39. M. Vahter, L. Frieberg, B. Rahnster, A. Nygren, and P. Nolinder. Airborne arsenic and urinary excretion of metabolites of inorganic arsenic among smelter workers. *Int. Arch. Occup. Environ. Health* **57:** 79–91 (1986).
40. X.C. Le, M. Mingsheng, and A.W. Norris. Speciation of arsenic compounds using high-performance liquid chromatography at elevated temperature and selective hydride generation atomic fluorescence detection. *Anal. Chem.* **68:** 4501–4506 (1996).
41. M.L. Cervera, R. Montoro, J.E.S. Uria, A.M. Garcia, and A.S. Medel. Determination of arsenic in foods by ashing, tandem on-fine continuous separation, and ICP-OES analysis. *Atom. Spectrosc.* **16:** 139–44 (1995).
42. P. Thomas and K. Sniatecki. Inductively coupled plasma mass spectrometry: application on the determination of arsenic species.

Fres. J. Anal. Chem. **351:** 410–14 (1995).
43. N. Ybanez, M.L. Cervera, R. Montoro, and M. De La Guardia. Comparison of dry mineralization and microwave-oven digestion for the determination of arsenic in mussel products by platform in furnace Zeeman-effect atomic absorption spectrometry. *J. Anal. Atom. Spectrom.* **6:** 379–84 (1991).
44. J. Alberti, R. Rubio, and G. Rauret. Arsenic speciation in marine biological materials by LC–UV-HG–ICP-OES. *Fres. J. Anal. Chem.* **351:** 415–19 (1995).
45. E.A. Elkhatib, D.L. Bennet, and R.J. Wright. Arsenic sorption and desorption in soils. *Soil. Sci. Soc. Am. J.* **48:** 1025–30 (1984).
46. E.H. Larsen, G. Pritzl, and S.H. Hansen. Arsenic speciation in seafood samples with emphasis on minor constituents. An investigation by high performance liquid chromatography with inductively coupled plasma mass spectrometric detection. *J. Anal. Am. Spectrom.* **8:** 1075–83 (1993).
47. S.A. Mathes, R.F. Farell, and A.J. Mackie. A microwave system for the acid dissolution of metal and mineral samples. *J. Technol. Prog. U.S. Bur. Mines Tech. Prog. Rep.* **120** (1983).
48. W. Holak and J. Spechio. Determination of total arsenic, As(III) and As(V), in foods by atomic absorption spectrometry. *Atom. Spectrosc.* **12:** 105–108 (1991).
49. N. Thiex. Solvent extraction and flameless atomic absorption determination of arsenic in biological materials. *J. Assoc. Off. Chem.* **63:** 496–99 (1980).
50. A. Lebouil, S. Notelet, A. Cailleux, A. Tutcant, and P. Allain. Determination of arsenic in urine after separation of arsenobetaine. *Toxicorama* **9:** 171–76 (1997).
51. S. Bransh, L. Ebdon, P. O'Neil. Determination of arsenic species in fish by directly coupled high performance liquid chromatography–inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **9:** 33–37 (1994).
52. J. Alberti, R. Rubio, and G. Rauret. Extraction method for arsenic speciation in marine organisms. *Fres. J. Anal. Chem.* **351:** 420–25 (1995).
53. A. El Moll, R. Heimburger, F. Lagarde, M.J.F. Leroy, and E. Maier. Arsenic speciation in marine organisms: from the analytical methodology to the constitution of reference materials. *Fres. J. Anal. Chem.* **354:** 550–56 (1996).
54. P. Bavazzano, A. Perico, K. Rosendahl, and P. Apostoli. Determination of urinary arsenic by solvent extraction and electrothermal atomic absorption spectrometry. A comparison with directly coupled high-performance liquid chromatography–inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **11:** 521–24 (1996).
55. X. Zhang, R. Cornelis, J. De Kimpe, and L. Mees. Arsenic speciation in serum of uremic patients based on liquid chromatography with hydride generation atomic absorption spectrometry and on-fine UV photo-oxidation digestion. *Anal. Chim. Acta* **319:** 177–85 (1996).
56. D. Heitkemper, J. Creed, J.A. Caruso, and F.L. Fricke. Speciation of arsenic in urine using high-performance liquid chromatography with inductively coupled plasma mass spectrometric detection. *J. Atom. Spectrom.* **4:** 279–84 (1989).
57. B.S. Sheppard, J.A. Caruso, D.T. Heitkemper, and K.A. Wolnik. Arsenic speciation by ion chromatography with inductively coupled plasma mass spectrometric detection. *Analyst* **117:** 971–75 (1992).
58. B.S. Sheppard, W.L. Shen, J.A. Caruso, D.T. Heitkemper, and F.L. Frick. Elimination of argon chloride interference on arsenic speciation in inductively coupled plasma mass spectrometry using ion chromatography. *J. Anal. Atom. Spectrom.* **5:** 431–35 (1990).
59. D.T. Heitkemper, J.T. Creed, and J.A. Caruso. Helium microwave-induced plasma mass spectrometric detection for reversed-phase high-performance liquid chromatography. *J. Anal. Atom. Spectrom.* **4:** 285–93 (1989).
60. C.J. Hwang and S.J. Jiang. Determination of arsenic compounds in water samples by liquid chromatography–inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* **289:** 205–213 (1994).
61. M. Lopez-Gonzalves, M.M. Gomez, M.A. Placios, and C. Càmara. Urine clean-up method for determination of six arsenic species by LC–AAS involving microwave assisted oxidation an hydride generation. *Chromatographia* **43:** 507–512 (1996).
62. K.A. Francesconi, R.V. Stick, and J.S. Edmonds. Glyceryl-phosphoryl arsenocholine and phosphatidylarsenocholine in yellow mullet (Aldrichetta forsteri) following oral administration of arsenocholine. *Experientia* **46:** 464–66 (1990).
63. Y. Shibata and M. Morita. Exchange of comments on identification and quantification of arsenic species in dogfish muscle reference material for trace elements. *Anal. Chem.* **61(2):** 6–18 (1989).
64. A. Christakopoulus and H. Norin. Cellular metabolism of arsenocholine. *J. Appl. Toxicol.* **8:** 219–24 (1988).
65. J.K. Lamble and S.J. Hill. Arsenic speciation in biological samples by on-fine high performance liquid chromatography–microwave digestion-hydride generation-atomic absorption spectrometry. *Anal. Chim. Acta* **334:** 261–70 (1996).
66. E.H. Larsen. Speciation of dimethylarsinyl-riboside derivatives (arsenosugars) in marine reference materials by HPLC–ICP-MS. *Fres. J. Anal. Chem.* **352:** 382–88 (1995).
67. U. Ballin, R. Kruse, and H.A. Rüssel. Determination of total arsenic and speciation of arsenobetaine in marine fish by means of reaction-headspace gas chromatography utilizing flame-ionisation detection and element specific spectrometric detection. *Fres. J. Anal. Chem.* **350:** 51–61 (1994).
68. K. Dix, C.J. Cappon, and T.Y. Toribara. Arsenic speciation by capillary gas–liquid chromatography. *J. Chromatogr. Sci.* **25:** 164–69 (1987).
69. F.T. Henry and T.M. Thorpe. Gas chromatography of the trimethylsilyl derivatives of arsenic, arsenious and dimethylarsinique acids. *J. Chromatogr.* **166:** 577–86 (1978).
70. M. Albert, C. Demesmay, and J.L. Rocca. Analysis of organic and non-organic arsenious or selenious compounds by capillary electrophoresis. *Fres. J. Anal. Chem.* **351:** 426–32 (1995).
71. S.S. Goyal, A. Hafez, and D.W. Rains. Simultaneous determination of arsenite, arsenate, selenite and selenate in waters using suppressed ion chromatography with ultraviolet absorbance detection. *J. Chromatogr.* **537:** 269–76 (1991).
72. H. Ding, J. Wang, L.G. Dorsey, and J. Caruso. Arsenic speciation by micellar liquid chromatography with inductively coupled plasma mass spectrometric detection. *J. Chromatogr. A* **694:** 425–31 (1995).
73. M. Morita, T. Uehiro, and K. Fuwa. Determination of arsenic compounds in biological samples by liquid chromatography with inductively coupled argon plasma-atomic emission spectrometric detection. *Anal. Chem.* **53:** 1806–1808 (1981).
74. C. Demesmay, M. Olle, and M. Porthault. Arsenic speciation by coupling high-performance liquid chromatography with inductively coupled plasma mass spectrometry. *Fres. J. Anal. Chem.* **348:** 205–210 (1994).
75. P. Morin, M.B. Amran, S. Favier, R. Heimburger, and M. Leroy. Speciation of arsenical species by ion-exchange and ion pair reversed phase liquid chromatography. *Fres. J. Anal. Chem.* **339:** 504–509 (1991).
76. P. Morin, M.B. Amran, M.D. Lakkis, and M.J.F. Leroy. Ion-pair reversed-phase liquid chromatography of arsenic species on polymeric styrene-divinylbenzene packed columns with an alkaline aqueous mobile phase. *Chromatographia* **33:** 581–85 (1992).
77. P. Teräsahde, M. Pantsar-Kallio, and P.K.G. Manninen. Simultaneous determination of arsenic species by ion chromatography-inductively coupled plasma mass spectrometry. *J. Chromatogr. A* **750:** 83–88 (1996).
78. F. Lagarde, A. Hagege, and M. Leroy. Utilization of HPLC–ICP-AES and HPLC–HG-QFAAS couplings for speciation: application in food products. *Analusis* **25:** 31–34 (1997).
79. C.F. Poole and S.K. Poole. *Chromatography Today*. Elsevier Sciences Publishers B.V., Arnsterdam, The Netheerlands, 1991, pp 411–22.
80. D. Beauchemin, K.W.M. Siu, J.W. Mclaren, and S.S. Berman.

Determination of arsenic species by high-performance liquid chromatography inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **4:** 285–89 (1989).
81. V.A. Fassel, G.W. Rice, and K.E. Laurance. Ultrasonic nebulization of liquid samples for analytical inductively coupled plasma-atomic spectroscopy. *Anal. Chem.* **56:** 289–94 (1984).
82. K.J. Irgolic, R.A. Stockton, and D. Chakraborti. Determination of arsenic and arsenic compounds in water supplies. *Spectrochim. Acta* **38:** 437–42 (1983).
83. K.E. Lafreniere, V.A. Fassel, and D.E. Eckels. Elemental speciation via high performance liquid chromatography combined with inductively coupled plasma atomic emission spectroscopic detection: application of a direct injection nebulizer. *Anal. Chem.* **59:** 879–87 (1987).
84. F.E. Brickman, K.L. Jewett, and W.P. Iverson. Graphite fumace atomic absorption spectrophotometers as automated element-specific detectors for high-pressure liquid chromatography. The de-termination of arsenite, arsenate, methylarsinic acid and dimethylarsinic acid. *J. Chromatogr.* **191:** 31–46 (1980).
85. E.H. Larsen and S.H. Hansen. Separation of arsenic species by ion-pair and ion exchange high performance liquid chromatography. *Mitrochim. Acta* **109:** 47–51 (1992).
86. K.A. Francesconi, P. Micks, R.A. Stockton, and K.J. Irgolic. Quantitative determination of arsenobetaine the major water-soluble arsenical in three species of crabs using high pressure liquid chromatography and inductively coupled argon plasma emission spectrometer as the arsenic specific detector. *Chemosphere* **14:** 1443–53 (1985).
87. T. Maitani, S. Uchiyama, and Y. Saito. Hydride generation-flame atomic absorption spectrometry as an arsenic detector for high performance liquid chromatography. *J. Chromatogr.* **391:** 161–68 (1987).
88. F.E. Pick, P.R. De Beer, S.M. Prinsolo, and L.P. Van Dyk. Contaminant and formulation analysis of MSMA using high-pressure liquid chromatography–graphite furnace atomic absorption spectrometry. *Pesticide Sci.* **21:** 45–49 (1987).
89. R. Rubio, A. Padro, J. Alberti, and G. Rauret. Speciation of organic and inorganic arsenic by HPLC–HG-ICP/OES. *Microchim. Acta* **109:** 39–45 (1992).
90. R. Rubio, A. Padro, and G. Rauret. LC–HG-QFAAS versus LC–HG-ICP-/OES in arsenic speciation. *Fres. J. Anal. Chem.* **351:** 331–33 (1995).
91. Y. Shibata and M. Morita. Speciation of arsenic by reversed-phase high performance liquid chromatography–inductively coupled plasma mass spectrometry. *Anal. Sci.* **5:** 107–109 (1989).
92. D. Velez, N. Ybanez, and R. Montoro. Optimisation of the extraction and determination of monomethylarsinic and dimethylarsinic acids in seafood products by coupling liquid chromatography with hydride generation atomic absorption spectrometry. *J. Anal. Atom. Spectrom.* **11:** 271–77 (1996).
93. B.S. Chana and N.J. Smith. Urinary arsenic speciation by high-performance liquid chromatography–atomic absorption spectrometry for monitoring occupational exposure to inorganic arsenic. *Anal. Chim. Acta* 177–86 (1987).
94. H. Heng-Bin, L. Yan-Bing, M. Shi-Fen, and N. Zhe-Ming. Speciation of arsenic by ion chromatography and off-line hydride generation electrothermal atomic absorption spectrometry. *J. Anal. Atom. Spectrom.* **8:** 1085–90 (1993).
95. J. Stummeyer, B. Harazim, and T. Wippermam. Speciation of arsenic in water samples by high performance liquid chromatography–hydride generation at trace levels using a post-column reaction system. *Fres. J. Anal. Chem.* **354:** 344–51 (1996).
96. C.T. Tye, S.J. Haswell, P. O'Neill, and K.C.C. Bancroft. High performance liquid chromatography with hydride generation/atomic absorption spectrometry for the determination of arsenic species with application to some water sample. *Anal. Chim. Acta* **169:** 195–98 (1985).
97. R. Rubio, G. Rauret, I. Peralta, and J. Alberti. Separation of arsenic species by HPLC–ICP/DES arsenocholine behavior. *J. Liq. Chromatogr.* **16:** 3531–37 (1993).
98. J. Szpunar-Lobinka, C. Write, R. Lobinski, and F.C. Adams. Separation techniques in speciation analysis for organometallic species. *Fres. J. Anal. Chem.* **351:** 351–77 (1995).
99. E.C.V. Butler. Determination of inorganic arsenic species in aqueous samples by ion-exclusion chromatography with electrochemical detection. *J. Chromatogr.* **450:** 353–60 (1988).
100. P. Boucher, M. Accominotti, and J.J. Vallon. Arsenic speciation by ion pair reversed-phase liquid chromatography with coupled amperometric and ultraviolet detection. *J. Chromatogr. Sci.* **34:** 226–29 (1996).
101. A.W. Boorn and R.F. Browner. Effects of organic solvents in inductively coupled plasma atomic emission spectrometry. *Anal. Chem.* **54:** 1402–1410 (1982).
102. P.W. Boumans. *Inductively Coupled Plasma Emission Spectrometry. Part I: Methodology Instrumentation and Performance,* P.W.J.M. Boumans, Ed. Awiley-Intersciences Publication, New York, NY, 1987, pp 45–68.
103. G. Rauret, R. Rubio, and A. Padro. Arsenic speciation using HPLC–HG-ICP/AES with gas–liquid separator. *Fres. J. Anal. Chem.* **340:** 157–60 (1991).
104. W. Nisamaneepong, M. Ibrahim, and T.W. Gilbert. Speciation of arsenic and cadmium compounds by reversed-phase ion pair liquid chromatography with single wavelength inductively coupled plasma detector. *J. Chromatogr. Sci.* **22:** 473–79 (1984).
105. S.C.K. Shum and R.S. Houk. Elemental speciation by anion exchange and size exclusion chromatography with detection by inductively coupled plasma mass spectrometry with direct injection nebulization. *Anal. Chem.* **65:** 2972–76 (1993).
106. S.A. Pergantis, E.M. Heitmar, and T.A. Hinners. Microscale flow injection and microbore high performance liquid chromatography coupled plasma mass spectrometry via high efficiency nebulizer. *Anal. Chem.* **65(4):** 30–35 (1995).
107. W.R. Cullen and M. Dodd. The photo-oxidation of solutions of arsenicals to arsenate: a convenient analytical procedure. *App. Organometr. Chem.* **2:** 1–7 (1988).
108. E.H. Larsen. Electrothermal atomic absorption spectrometry of inorganic and organic arsenic species using conventional and fast furnace. *J. Anal. Atom. Spectrom.* **6:** 375–77 (1991).
109. I.D. Brindle, H. Alarabi, S. Karshman, X.C. Le, and S. Zheng. Combined generator/separator for continuous hydride generation: application to on-fine pre-reduction of arsenic(V) and determination of arsenic in water by atomic emission spectrometry. *Analyst* **117:** 407–11 (1992).
110. G. Horlick, S.H. Tan, M.A. Vaughan, and Y. Shao. *Inductively Coupled Plasma in Analytical Atomic Spectrometry.* Montaser and D.W. Golightly, Ed. VCH Publishers, New York, NY, 1987, pp 361–98.
111. K.E. Jarvis, A.L. Gray, and R.S. Houk. *Handbook of Inductively Coupled Mass Spectrometry*. Blakie Academic and Professional, Glascow, Scotland, 1992.
112. S.A. Pergantis, E.M. Heithmar, and T.A. Hinners. Speciation of arsenic animal feed additives by microbore high-performance liquid chromatography with inductively coupled plasma mass spectrometry. *Analyst* **122:** 1063–68.(1997).
113. R.C. Hutton. *The Choice of Acid Matrix in ICP-MS*. VG Isotopes, Winsford, U.K., 1986.
114. H. Garraud, A. Woller, P. Fodor, and O.F.X. Donard. Trace elemental speciation by HPLC using microbore columns hyphenated to atomic spectrometry. *Analusis* **25:** 25–31 (1997).

Manuscript accepted July 29, 1999.