Toxic arsenic compounds in environmental samples: Speciation and validation

M. Leermakers, W. Baeyens, M. De Gieter, B. Smedts, C. Meert, H.C. De Bisschop, R. Morabito, Ph. Quevauviller

Speciation of arsenic (As) compounds in environmental samples requires rigorous analytical procedures at each stage of sample collection, treatment and measurement. In view of the very high number of As species occurring in environmental samples, this article deals with only those that are considered toxic, including As-containing warfare agents and arsenobetaine (AB), because this is the most abundant As species in marine fish. With the exception of the tetramethylarsonium ion, acute toxicity generally decreases with increasing degree of methylation. The warfare agents considered are mostly phenylarsenic compounds.

During storage, events, such as changes in oxidation state or induced by microbial activity or losses by volatilization or adsorption, have to be avoided. Extraction of arsenicals from soils and sediments is still a critical step that is not very well controlled. As long as marine animals are considered, extraction efficiencies >90% are commonly obtained [K.A. Francesconi, D. Kuehnelt, Analyst (Cambridge, UK) 129 (2004) 373]. This is logical, since marine animals are rich in AB, and this small molecule is soluble in water and methanol and in mixtures of them. In terrestrial organisms and plants, extraction efficiencies can be much lower and vary according to the extraction conditions.

Since most environmental As species are generally present in soluble forms, liquid-separation techniques, first high-performance liquid chromatography (HPLC) but also capillary electrophoresis (CE), are most frequently used for separating As species. Fast separations can be performed with narrow-bore reversed-phase HPLC columns. Pre-column derivatizations with hydride generation [e.g., As(III), As(V), MA, DMA] and mercaptans/dimercaptans (e.g., Clarks I and II and Pfiffikus) allow separation of the arsenicals by gas chromatography (GC).

The most popular detectors are inductively coupled mass spectrometry (ICP–MS) and atomic fluorescence spectrometry (AFS), especially after HPLC separation and hydride formation, increasingly replacing atomic absorption spectrometry (AAS), while HPLC–MS or HPLC–MS–MS (HPLC–MS2) is the detector of choice for (less toxic) arsenosugars and arsenolipids. For phenylarsenic warfare species, ultraviolet (UV) detection is also still used frequently.

Finally, as other measurement fields, speciation analysis requires the availability of suitable reference materials (RMs) for verification of accuracy and quality assurance. We address these important aspects although the number of certified RMs is rather limited.

Keywords: Arsenic; Arsenobetaine; Calibrant; Certified reference material; Chromatography; Detection; Environmental sample; Extraction; Sampling; Speciation; Storage; Validation; Warfare agents

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1. Introduction

Although a toxic element like arsenic (As) has for a long time been regarded upon as only an accidental contaminant, it is widely distributed in the environment, both because of natural sources as through anthropogenic applications. The As content of the Earth's crust averages 2.5 mg/kg [2]. The highest amounts of mineral As generally occur in ores, mainly sulphides, as As is chalcophilic. As can be associated with these ores as a minor

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compound [e.g., in pyrite (FeS_2) and sphalerite (ZnS)] or a major compound [e.g., in arsenopyrite (FeAsS), orpiment (As_2S_3) and realgar (As_4S_4)]. However, As has numerous applications in industry and agriculture (e.g., in the glass and semiconductor industries, and as pesticide, herbicide, growth promoter for swine, food additive to combat deceases in poultry or preservative for wooden structures, such as pilings and docks, on the coast).

From the First World War onwards, organo-arsenicals were developed, stockpiled and also used as harassing, vomiting or vesicant agents in chemical warfare [3]. After Word War II, a large part of the stockpile was deposited on land or dumped at sea [4]. However, As warfare agents continued to be produced and stored by both countries of the former Soviet Union and the USA as part of their chemical-weapons stockpiles. These are currently in a process of being destroyed in an environmentally safe manner, as requested by the Convention on Chemical Weapons [5]. Although old or abandoned chemical shells, bombs or bulk containers are confined to specific regions (e.g., Europe, China, and Japan), significant amounts of these toxic arsenicals still remain in the environment.

As has several oxidation states (+III, +V, 0, −III) and a variety of inorganic and organic forms. Fig. 1 shows the forms of As that are most widespread in the environment (thus not the warfare agents). Extensive toxicity studies of As have shown that different forms exhibit different toxicities. Of all the species listed in Fig. 1, inorganic As species are more toxic than organic compounds. With the exception of the tetramethylarsonium (TeMA) ion, acute toxicity generally decreases with increasing degree of methylation (Table 1).

Inorganic forms can be found in small quantities in living organisms, but generally organoarsenic species dominate. The presence of organoarsenicals in marine life was confirmed in 1977 when arsenobetaine (AB) was identified in western rock lobster. Since then, a large number of organoarsenic species have been identified in marine tissues. While AB is the major species in fish and seafood, they can also contain minor concentrations of inorganic As, methylated compounds, arsenocholine

Table 1. Experimental LD_{50} values of As species (adapted from [6])

Arsenic species	LD_{50} (g/kg)
As(III)	0.0345
MMA	1.8
DMA	1.2
TeMA	0.89
TMAO	10.6
AsC	>6.5
AsB	>10.0

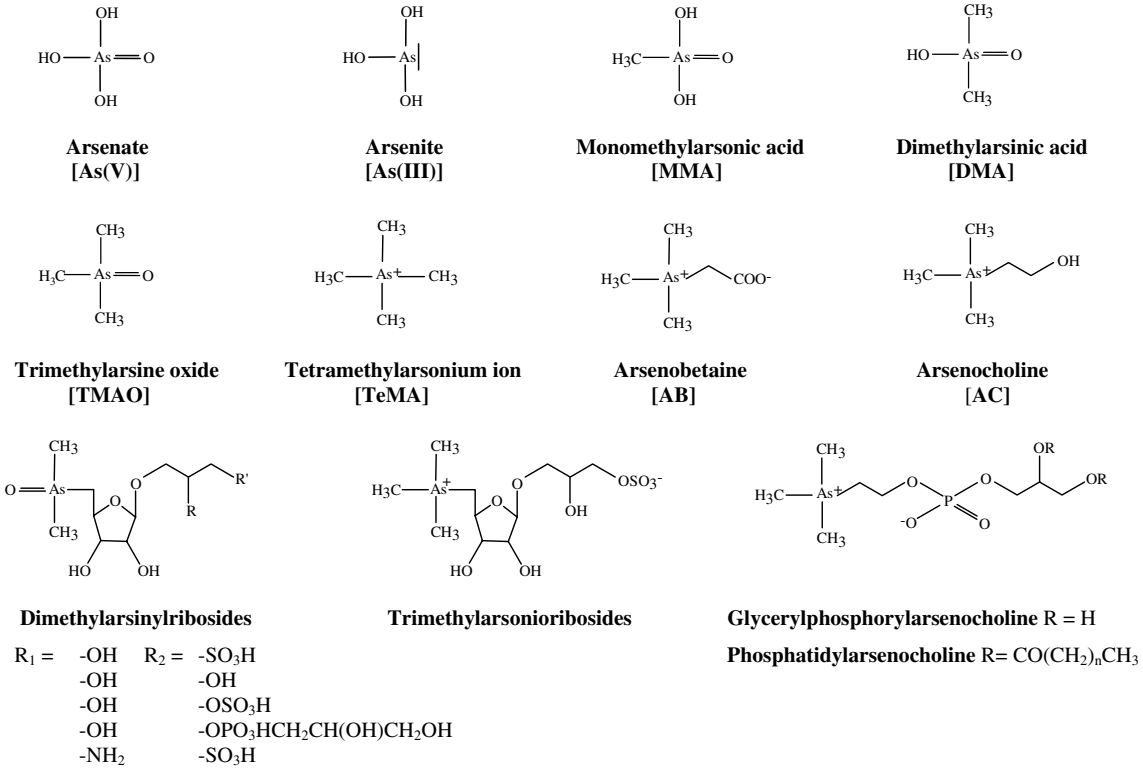

Figure 1. Structures of the most common As compounds in the marine environment.

(AC^+) or arsenosugars. However, marine organisms are known for their rich metabolism, so we presume that there are more organoarsenicals yet to be characterized.

Few assays of toxicity have yet been made for arsenolipids. Knowledge of the toxicity of arsenosugars is also limited. Kaise et al. [7] investigated the cytotoxicity of the arsenosugar 1-(2′,3′-dihydroxypropyl)-5-desoxyribosyldimethyl-arsine oxide on mammalian cell cultures. The compound was found to exhibit an acute toxicity 1/2800th of that of sodium arsenite and 1/300th of that of sodium arsenate.

In contrast to the As compounds listed in Fig. 1, phenylarsenic compounds are, to our knowledge, not the result of natural processes, but have been produced and used as chemical-warfare agents. The most important are listed in Table 2. Clark I, Clark II, and Adamsite are strong irritants, called ''sternutators''. Toxic effects of these compounds occur from concentrations in air of approximately 0.1 mg/m^3. Pfiffikus is toxic by inhalation (irritant) and by skin contact, and Lewisite is a potent blister agent.

From this point of view, the need for adequate, sensitive and accurate speciation methods for As is self-evident. In this article, we will focus on As(III), As(V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), AB and phenylarsenic agents mentioned in Table 2.

The very first report of As species separation was the method of Braman and Foreback [8], which was based on the ability of As species to form volatile hydrides and on their differences in boiling points (Fig. 2). The vapor-phase hydrides (arsines) formed were then concentrated by cryogenic trapping and successively released from the trap by gradually increasing the temperature. Increased awareness of the overall abundance of As in the environment, occupational exposure to As and its toxicity have led to the need for adequate, and especially more sensitive, analytical techniques for As speciation. This quest for improved analytical methods has led to the development of techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), hyphenated to detectors, such as mass spectrometry (MS), inductively coupled plasma MS (ICP–MS), ICP atomic emission spectrometry (ICP–AES), graphite furnace atomic absorption spectrometry (GF–AAS), atomic fluorescence spectrometry (AFS), electron capture detection (ECD) and ultraviolet (UV). Limits of detection (LODs) have decreased to present-day levels of only some tens of μg/L As for each species.

Table 2. Warfare compounds

Name	Formula	(Abbreviation) Mil. code
Diphenylarsine chloride	Ph_2AsCl	Clark I
Diphenylarsine cyanide	Ph_2AsCN	Clark II
Phenarsazine chloride	$Ph_2As(NH_2)Cl$	Adamsite
Diphenylarsine dichloride	Ph_2AsCl_2	Pfiffikus
2-Chlorovinylarsine dichloride	Cl-vinyl$AsCl_2$	Lewisite

2. Calibrants for As speciation

Pure As(III), As(IV), MMA, DMA, AB and phenylarsonic acid (AsPhen) are commercially available.

Analytical calibrating standards for the Clarks and Pfiffikus can be synthesized from $AsCl_3$ (CAS No. 7784-34-1) and triphenylarsine (CAS No. 603-32-7) [9].

The synthesized organo-arsenicals [phenyldichloroarsine and diphenylchloroarsine and diphenylcyanoarsine (Clark II)] were isolated and purified by distillation under reduced pressure. Synthesized compounds are dissolved in acetone or ethyl acetate and characterized via MS and infrared (IR) spectra in acetone or ethylacetate. Purity levels for the hydrolysates, the main forms in methanolic or aqueous solutions, are above 90%.

For Adamsite (phenarsazine chloride), the following synthesis reactions can be applied (e.g., Sartori [9]):

(a) reaction of diphenylamine with As trichloride;
(b) heating diphenyl hydrazine with As trichloride; and,
(c) boiling aniline with As trichloride, addition of sodium hydroxide and treatment of the obtained oxide with hydrochloric acid.

Lewisite can be produced by Friedel Craft's alkylation of As(III) chloride with ethane [10].

$$(CH_3)_nAsO(OH)_{3-n} + H^+ + BH_4^- \rightarrow (CH_3)_nAs(OH)_{3-n} + H_2O + BH_3$$

$$(CH_3)_nAs(OH)_{3-n} + (3-n)H^+ + (3-n)BH_4^- \rightarrow (CH)_nAsH_{3-n} + (3-n)H_2O + (3-n)BH_3$$

As compound	Reaction product	Boiling point
Arsenite H_3AsO_4	AsH_3	-55°C
Monomethylarsonic acid $CH_3AsO(OH)_2$	CH_3AsH_2	2°C
Dimethylarsinic acid $(CH_3)_2AsOOH$	$(CH_3)_2AsH$	36°C
Trimethylarsine oxide $(CH_3)_3AsO$	$(CH_3)_3As$	70°C

Figure 2. Arsine generation and corresponding boiling points (for the structure of the compounds, see Fig. 1).

3. Sampling, storage and chemical modification

For As, contamination will occur only rarely, as long as trace-element standard procedures are respected; it is rather the preservation of samples that will be one of the troublesome steps in As-speciation analysis. Events, such as changes in oxidation state, changes induced by microbial activity or losses by volatilization or adsorption, have to be avoided.

Aqueous samples intended for total As determination have been observed not to be subject to losses during storage, when kept in acid-washed glass, PTFE or polyethylene containers [11]. However, regarding storage for As-speciation experiments, there is limited information available on appropriate storage conditions for As.

An overview of the influence of critical factors for species stability (pH, temperature, light and container material) and of procedures for the preservation of the integrity of species is given in Ariza et al. [12]. Procedures recommended are freezing, cooling, acidification, sterilization, deaeration, addition of ascorbic acid and/or storage in the dark. However, there is no general agreement on these procedures and reports sometimes even conflict. This is especially true for complex solid matrices, such as soils, sediments and biological tissues. Nonetheless, for samples where bacteria will exist naturally, storage at low temperatures or even freeze-drying [13,14] is required to prevent biological activity from modifying the nature of the sample.

For aqueous samples, time and temperature studies provided information that, at higher concentrations (20 μg/L), immediate storage of filtered (0.45 μm) natural waters, at about 5°C, will preserve As(III) and As(V) concentrations for about 30 days [15]. It is advised that samples with lower As concentrations be kept in the dark, at 4°C [16]. As reported above, in aqueous or methanolic solutions, the phenylarsenic compounds can be hydrolyzed (hydroxide or oxide from) and oxidized (acid form) (e.g., see Fig. 3).

In an overview paper, Francesconi and Kuehnelt [1] also addressed the stability of As species during extraction and chromatography steps.

4. Extraction

Most present-day techniques allow the assessment of As species in aqueous samples, without requiring any preconcentration or pretreatment. This is particularly true when coupled to hydride generation (HG), which has been shown to significantly increase the sensitivity of the analytical method, due to the high selectivity of hydride formation and separation of the gaseous arsines from the matrix. However, this is not true for seawater samples because the chlorides interfere during the separation processes and/or the hydride formation. Speciation of As species in seawater was performed by collecting these species on cobalt (III) oxide powder [17] with or without

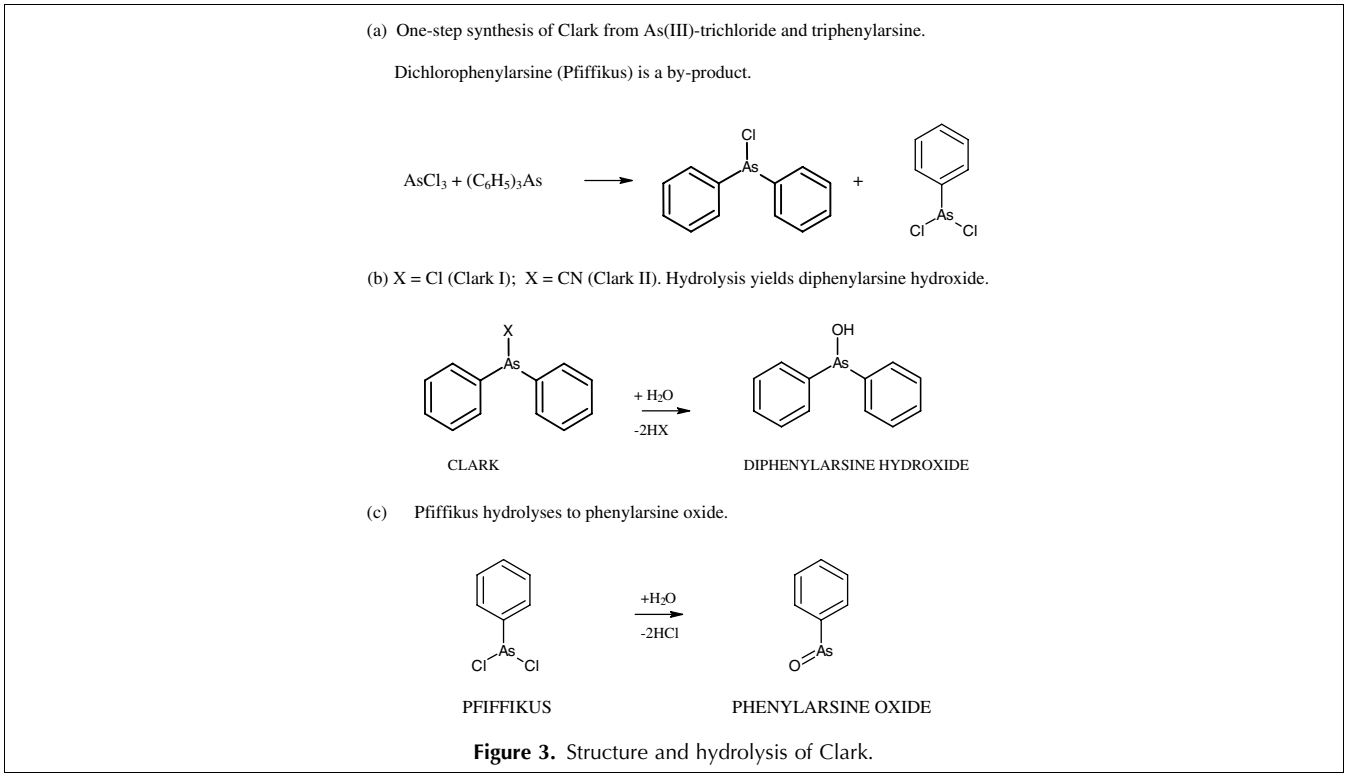

Figure 3. Structure and hydrolysis of Clark.

prior benzene extraction. Almost 100% of As(III) and As(V) in an aqueous solution can be collected on cobalt (III) oxide powder when the pH of the solution is between 1.0 and 11.0. After addition of potassium iodide to the seawater, DMA is extracted with benzene, then the solution is acidified with 7.2 M HCl, and DMA is collected on cobalt (III) oxide powder. However, it was impossible to extract AB from seawater with benzene, so AB was first decomposed into inorganic As through acid decomposition (HNO_3–$HClO_4$–H_2SO_4 mixed acid, 350°C), then the solution was further treated as in the determination of As(III) and As(V) (as above).

Sediment or soil samples may be treated in different ways, depending on the information required. Water contained in sediments can be removed and analyzed as described previously, but speciation information on the solid fraction is more difficult to acquire. For some time, series of sequential extractions have been employed to acquire the information needed to understand the cycling of As in sediments (e.g., on water-soluble, phosphate-exchangeable, organically bound and residual phases in such media [18,19]).

Literature on extraction procedures for detection, hyphenated to chromatographic techniques, is much more recent; extractions of sediments with phosphoric acid and/or hydroxylamine hydrochloride have allowed HPLC measurement of labile As, since they preserve the two redox states of As in this fraction [20]; Garcia-Manyes et al. [21] reported a similar procedure with phosphoric acid and ascorbic acid; Montperrus et al. [22] efficiently extracted As from sediment and sludge using orthophosphoric acid, but reported a higher efficiency from old formation soils, with oxalate. Other extraction chemicals employed are methanol/hydrochloric acid/water [23], acetone and hydrochloric acid [24] and 1,3-propanedithiol or 1,2-ethanedithiol [25].

Classical methods for extracting phenylarsenic compounds from sediments or soil (shaking, sonication or Soxhlet) have been tested [26,27], yielding relatively low recoveries (highest 40% with Soxhlet [14]).

Supercritical fluid extraction with carbon dioxide and methanol with 15% dichloromethane as additional modifier have yielded a maximum extraction efficiency of 40%, depending on temperature and pressure. The highest extraction efficiencies (nearly 100%) for phenylarsenic compounds were obtained with a combination of thermal desorption and GC–MS [14], as indicated by repeated measurements with spiked and real soil samples. Extraction of arsenicals from soils and sediments is still a critical, not very well-controlled step.

Smedts et al. [28] dissolved sub-samples from different munition shells, containing TNT and phenylarsenic compounds, in acetone (qualitative GC–MS analysis) or methanol (quantitative HPLC or MEKCl analysis).

For biological materials, several extraction methods for As speciation have been developed, focusing on lower solvent volumes and reduced extraction times,. Biological tissues with high fat content may need to be defatted prior to extraction of As. Solvents, such as acetone or ether, have been used for this purpose [29,30]. Extraction of solid samples is almost always aided by techniques such as shaking, heating or sonication. The latter is most popular, as the dispersion of tissue is thought to be maximized. For extraction from biological materials, mixtures of methanol/water or methanol/chloroform are used most often [31], but, due to the difficulty in handling chloroform, the water/methanol mixture is widely applied.

McSheehy et al. [32] compared the effects of three solvent systems (water, methanol and 1:1 methanol–water) on both the species and the total amount of As extracted from molluscs. The reported data showed no difference between the various solvent systems. In fact, as long as marine animals are considered, extraction efficiencies >90% are commonly obtained [1]. This is logical, since marine animals are rich in AB, and this small molecule is soluble in water and methanol and in mixtures of them. Marine algae present a slight tougher assignment, and often <80% of the total As is extracted [1]. In terrestrial organisms and plants, extraction efficiencies can be much lower and variable according to the extraction conditions.

An alternative possibility is enzymatic digestion with trypsin, but the activity of the enzyme has to be monitored, as differences in activity can lead to poor reproducibility [33]. The extract itself may need further treatment prior to separation, since matrix components are also often co-extracted; clean-up of the extracts on a silica column proved to be a possible source of loss of compounds, while filtration with C_{18} or Florisil cartridges was observed to be efficient [16]. Solid particles that could damage chromatographic columns should also be removed.

5. Chromatographic separation

Since most environmental As species are generally present in soluble forms, liquid-separation techniques, in the first place HPLC but also CE, are the most frequently used for separating As species. Also, HPLC allows easy coupling with element-specific detectors; only a simple interface is required.

The chromatographic separation depends on pH. At neutral pH, arsenate (As(V), $pK_{a1} = 2.3$), MMA ($pK_a = 3.6$) and DMA ($pK_a = 6.2$) are present as anions; AC^+, trimethylarsine oxide (TMAO, $pK_a = 3.6$) and TeMA ion as cations; AB ($pK_a = 2.18$) as zwitterion; arsenite and (As(III), $pK_{a1} = 9.3$) as an uncharged species. As a result, both anion exchange (for separating As(V), As(III), MMA and DMA) and cation exchange (for separating AB, AC, TMAO and TeMA) are commonly used.

The use of reversed-phase ion-pair HPLC, with appropriate counter ions in the mobile phase (e.g., tetramethylammonium cation or heptanesulfonate) has also been documented; Le et al. [34] reported the separation of seven As species on a reversed-phase C18 column with a hexanesulfonate-containing mobile phase. Kohlmeyer et al. [35] – using anion exchange with nitric acid eluent together with a doubly-charged ion-pairing agent – and Sloth et al. [36] – using cation exchange and aqueous pyridine formate with gradient elution – respectively reported separations of 17 and 23 organoarsenicals in one chromatographic run.

Both studies presented contrasts in terms of reported matrix effects. Since they used different types of chromatography, Francesconi and Kuehnelt [1] suggested that a comparative study may reveal some novel fundamental processes.

A narrow-bore reversed-phase HPLC column with ion-pairing was used by Wangkarn and Pergantis [37] to separate As(III), As(V), MMA and DMA, which took about 2 min.

Overviews of different types of columns and mobile phases, applied in ion exchange and ion pair chromatography, are given in Gong et al. [38], McSheehy et al. [13] and Francesconi and Kuehnelt [1].

Nakazato et al. [39] obtained separation of As(V), As(III) and MMA on an ion-exclusion column packed with sulfonate polystyrene resin, with dilute nitric acid as mobile phase. The authors stated that this technique enabled robust, efficient separation of As in highly saline matrices, such as seawater or human urine. Indeed, other LC techniques have been widely used for separation of As species in all kinds of environmental samples, but reports on successful separation of As in seawater without prior treatment (see previous paragraph) appear to be rare because the presence of large amounts of chloride ions causes the separation performance to deteriorate.

Haas et al. [40] and Smedts et al. [28] separated various phenylarsenic compounds by HPLC, including diphenylarsine hydroxide (the hydrolyzed form of Clarks I and II), phenylarsine oxide (the hydrolyzed form of Pfiffikus) and triphenylarsine. Moreover, Haas et al. [40] also determined phenarsazine hydroxide (the hydrolyzed form of Adamsite). During method development, Haas et al. [40] tested two types of columns:

- on the RP-18 column, separation was achieved with an eluent gradient from 50/50 (v/v) to 95/5 (v/v) acetonitrile/water; and,
- on a cyanopropyl column, separation was achieved with an eluent gradient from 10/90 (v/v) to 70/30 (v/v) acetonitrile/water.

Smedts et al. [28] used a methanol/water eluent gradient on a reversed phase C-18 column, starting with 40/60 (v/v) and ending with 95/5 (v/v). The LOD was 10 times lower than that obtained by Haas et al. [40].

For biota samples, one-dimensional HPLC has often proved to be insufficient for baseline speciation of all As compounds, especially when As sugars were also included. Here, orthogonal HPLC or bi-dimensional chromatography can improve the speciation capacity of HPLC–ICP–MS.

Capillary zone electrophoresis (CZE) has been applied to the separation of inorganic and organoarsenic species (no phenylarsenic compounds) in standard solutions and drinking water by Morin et al. [41], Lopez-Sanchez et al. [42], and Corr and Anacleto [43]. However, because of matrix interference, the analysis of real samples appeared much more troublesome [44]. Phenylarsenic species (Clarks I and II and Pfiffikus) in retrieved munitions were analyzed by Smedts et al. [28] with CZE.

Although not as common as HPLC, some authors apply HG, based on the original method of Braman and Foreback [8], in conjunction with purge and trap GC (PT-GC). Volatile hydrides that are formed from reaction with sodium borohydride and hydrochloric acid are swept from solution and separated by GC. Slejkovec et al. [45] reported such a method of trapping the gaseous arsines in a liquid-cooled U-tube packed with Chromosorb W. Upon comparison with LC, the authors described a significant improvement in LODs due to the much larger sample volumes, up to 100 mL, whereas typically only 100 μL of sample is injected in LC. Separation of As compounds in gaseous emissions is a much more common application of GC; the compounds do not require additional derivatization prior to separation [46].

Haas et al. [40] carried out derivatization of Clarks I and II and Pfiffikus with mercaptans and dimercaptans. The total of diphenylarsenic and phenylarsenic compounds was then detected as the mercapto- and dimercapto-derivatives.

Schoene et al. [26] applied derivatisation with thioglycolic acid methyl ester prior to GC analysis, but low derivatisation yields were reported.

6. Detection methods

Over the years, three types of element-specific detectors that are especially suitable and sensitive have been used most often: ICP–MS; ICP–AES; and, GF–AAS. However, AFS and molecular detection modes, such as GC–MS, LC–MS or tandem MS (MS^2) are now growing in popularity. For phenylarsenic compounds, UV or UV diode array detection (DAD) is still common [28].

6.1. AAS

Until the 1980s, flame AAS (FAAS) was extensively used for As detection, but, because FAAS suffers from low sensitivity (LOD for As: 1 mg/L) and high background noise from the flame, GF-AAS was introduced. An improvement by a factor of 10–100 in analytical

sensitivity was obtained by using a small heated graphite tube. Most often, to exclude any interference, GF–AAS was combined with HG to detect total As. Additional research was conducted on the use of this technique in combination with HPLC. However, because of the long drying–ashing–atomizing cycle of GF–AAS, direct coupling to HPLC was found to be difficult.

Procedures of collecting chromatographic fractions, followed by batch analysis of each fraction, and on-line methods, in which effluent fractions are collected and periodically analyzed, have been developed [47]. Nevertheless, the method requires broad chromatographic peaks, because 30–60 s are needed for each determination.

Another interface, a flow-injection system using post-column HG, has resulted in real-time signals and LODs for As of low μg/L [48]. Anyhow, most probably due to these difficulties in coupling and the multi-element possibilities of techniques such as ICP, interest in AAS methods does not seem to be growing in the field of As detection, especially not in speciation.

6.2. ICP

Amongst the various methods of exciting elements for emission spectrometry, ICP is one of the most efficient. Indeed, it has found a widespread use in analytical techniques for As. In spite of MS being clearly the predominant mode of detection when coupled to the plasma method, there have also been some reports on AES being used.

Chausseau et al. [49] came to the conclusion that HPLC-ICP-AES may be a reliable technique for As speciation, when low LODs are not required; they obtained LODs ranging from 7 μg/L for As(III) to 18 μg/L for As(V). Lower values are not expected since the most sensitive line for As lies in the UV region, at 193.7 nm, and nearby, at 193.1 nm, a carbon emission line is present. Large amounts of organic matter in the sample will therefore interfere with As detection at 193.7 nm. The other As emission line (228.8 nm) also suffers from possible interferences (e.g., cadmium also has a strong emission line at this wavelength).

For many elements, the sensitivity of ICP–MS exceeds that of ICP–AES by more than two orders of magnitude; LODs for As are in the range of μg/L [50], but major spectroscopic and non-spectroscopic interferences are also encountered. The most obvious interference in the case of As is caused by the formation of $^{40}Ar^{35}Cl^{+}$ in the plasma and this interference is proportional to the concentration of Cl^{-} ions in the sample. Since As is monoisotopic, it is impossible to avoid this isobaric overlap with conventional quadrupole mass analyzers. Research in the field nevertheless revealed that the formation of $^{40}Ar^{35}Cl^{+}$ can be suppressed by injecting nitrogen into the plasma [51]. Alternatively, by making an additional coupling with HG between the chromatographic column and the detector, only arsines are carried to the detector, while the Cl^{-} ions remain in solution. However, this latter technique has the disadvantage that As species that do not form volatile arsines cannot be detected.

In recent years, a second generation of ICP–MS, with much higher resolution ($M/\Delta M > 10{,}000$ compared to >300 for low resolution) has been developed and widely accepted due to its outstanding performance characteristics. This high mass resolution ICP–MS technique allows the spectral separation of the $ArCl^{+}$ interference and the accompanying reduction in sensitivity results in LODs for routine As analyses of 0.7 μg/L.

Klaue and Blum [52] nevertheless compared ICP–MS in high-resolution mode to HG–ICP–MS in low-resolution mode. While ICP–MS allows determination of As above 1 μg/L, HG–ICP–MS resulted in an over 2000-fold increase in relative sensitivity; it offers LODs in the 0.2 ng/L range, so it enables sub-μg/L measurements of As in drinking water.

Despite the drawbacks of interferences, ICP–MS is extensively used as detector in As-speciation research. The flow rate of liquid samples through ICP is often similar to that through HPLC, so the combination of both instrumentations can easily be achieved, simply by connecting the effluent line of the column to the input line of the nebulizer. Nevertheless, HPLC column conditions are selected primarily to get optimal separation; but, of course, here too the limiting factors of ICP–MS must be taken into account (e.g., the use of phosphate buffer as mobile phase is not suitable for ICP–MS).

6.3. AFS

Research on improvement of LODs in As detection and speciation has resulted in AFS being optimized. The advantages of AFS over AAS have been described for a long time, both theoretically and experimentally [53,54]. However, it was not until recently that commercial AFS instruments were developed. Since then, AFS has become a promising technique for analyzing some environmentally important elements, including As.

The most widely used AFS systems are almost exclusively coupled to HG; indeed, as for AAS, HG eliminates light scattering and background interferences from the matrix, so it significantly increases the sensitivity of AFS.

There is in the literature one method not based on HG; the research group of Mester and Fodor [55] applied ultrasonic nebulization (USN) as the interface between HPLC and AFS, and achieved accurate separation and determination of As(III), As(V), MMA and DMA [56] and of AB and AC [55], down to absolute LODs of 8.9–50 ng (250 μL injection volume).

Several couplings of HPLC to HG–AFS have been applied. Gomez-Ariza et al. [57] described an anion-exchange HPLC–HG–AFS system for the speciation of As(III), As(V), DMA and MMA; they described LODs of 0.17, 0.38, 0.45 and 0.30 μg/L, respectively.

Arsenobetaine, a non-hydride forming As species, was also detected, by introducing on-line UV photo-oxidation prior to HG.

Other researchers, using cation-exchange HPLC coupled to UV–HG–AFS, have reported that AFS can provide analytical information on AB, AC, TMAO and TeMA [58]. However, the UV decomposition step in these HG–AFS procedures depends critically on the matrix and the As species. This implies that, for biological extracts, the standard addition method should be always used [1].

Le et al. [34] described on-line microwave derivatisation coupled to HPLC–HG–AFS; they studied the separation of 11 As compounds using ion-pair chromatography. This speciation technique was successfully applied in the study of arseno-sugars, which are often the common As-containing constituents in seaweed products.

All of these couplings were reported as being achieved easily. Due to this diversity and its high selectivity and sensitivity, AFS, together with ICP–MS, has become an important, promising technique for As speciation. Additionally, both techniques have been included in an extensive range of reports on different couplings and methods, and accurate determinations of As species in different environmental matrices (e.g., [1,13]).

6.4. GC–MS

MS is the most frequently used method for identifying and elucidating unknown compounds. Ionization of the compounds can be obtained by techniques such as ionspray, electrospray, atmospheric pressure chemical ionization (APCI), electron ionization (EI), and fast atom bombardment. Since most As compounds are not volatile, derivatization is necessary before GC separation.

Phenylarsenic compounds have been derivatized with mercaptans/dimercaptans [40] or thioglycolic acid methyl esters [14] before injection into the GC–MS, or directly injected after thermal desorption [14].

Smedts et al. [28] dissolved the phenylarsenic compounds in acetone before injecting them into GC–MS to check the purity of the standard solutions. Quantification by GC–MS is seldom required because As is monoisotopic, so isotope dilution is an option for only deuterated or ^{13}C organo-As compounds.

6.5. HPLC–MS or HPLC–MS^2

Recent articles on quantification with HPLC–MS have reported LODs below 30 ng/mL [59], approaching those of HPLC–ICP–MS. With MS^2, still better results can be obtained, since background noise is virtually absent in this mode.

Often HPLC–MS and HPLC–MS^2 are applied to characterize the numerous arsenicals, such as AB, AC and arsenosugars in biota-like algae [60,61], oysters [32] and clams [62].

When we focused on complex mixtures of phenyl-, diphenyl- and triphenylarsenic species, HPLC–MS or HPLC–MS^2 (EI and APCI) was applied [14], allowing the identification of additional organoarsenic compounds in soil and plant samples.

7. Certified reference materials

In the same way as other fields of measurement, As-speciation analysis requires suitable reference materials (RMs) to be available to verify accuracy and to meet quality-assurance needs [63]. Several interlaboratory comparisons were organized by the European Commission, through the BCR (French acronym for Community Bureau of Reference), between 1989 and 1995 to improve the state of the art in As-speciation analysis. The number of exercises needed to certify some As species in tuna fish and solutions illustrated the high degree of difficulty of this type of analysis [63]. The interlaboratory exercises took a step-wise approach involving six exercises of increasing difficulty, namely: solutions of six pure As species (arsenite As(III), arsenate As(V), MMA, DMA, AB and AC); solutions containing a mixture of the six As species; solutions containing the six As species together with intefering cations and anions; raw fish and mussel extracts; cleaned fish and mussel extracts; and, shark and mussel powders. The certification of total As, DMA and AB in tuna fish (in certified reference material BCR-627) was completed in 1996, along with the certification of AB in solution (BCR-626).

More recently, it was recognized as being necessary to produce environmental materials certified for a range of chemical forms of elements. In this respect, a project has been authorized to certify jointly butyltins, MeHg and As species in an oyster RM, BCR-710 [64]. The experience of this project has shown that, while acceptable comparability of results could be obtained for AB (certified at the levels (33 ± 7) mg/kg), the analytical state-of-the-art is still not sufficient to certify inorganic As species, AC and MMA (as there are insufficient data or the spread of results is too large) in a biological material. Regarding DMA, the agreement of results was not considered sufficient to achieve certification, and an indicative value was proposed instead [(0.82 ± 0.18) mg/kg].

As mentioned above, these three CRMs are the results of improvement schemes and/or have been produced in the frame of interlaboratory studies involving expert laboratories. They therefore represent the best the state-of-the-art As-speciation analysis and internationally-recognized references to establish traceability of measurements of the chemical forms of elements in various matrices.

Besides BCR, only the National Resource Council of Canada (NRCC) provides an RM for As speciation available on the market. However, this RM, dogfish

Table 3. List of certified reference materials available for As-speciation analysis

Matrix	BCR 626	BCR 627	BCR 710	DORM-2
	Solution	Tuna fish	Oyster tissue	Dogfish muscle
Total As (mg/kg)	–	4.8 ± 0.3	25.7 ± 2.7[a]	18 ± 1.1
DMA (mg/kg)	–	2 ± 0.3 μmol/kg	0.82 ± 0.18[a]	–
AsB (mg/kg)	1031 ± 6	52 ± 3 μmol/kg	33 ± 7	16.4 ± 1.1[a]
$TMAs^+$ (mg/kg)	–	–	–	0.248 ± 0.054[a]

[a]Indicative values only.

muscle, is certified only for total As and the concentration values of AB and TeMA ion are provided as indicative only.

Table 3 shows the CRMs available on the market. As can be seen, and as expected considering the importance of As species in the marine environment, all these materials are of marine origin. The only exception is BCR 626, AB solution; however, that was produced to support the quality of analysis of As species in tuna fish simultaneously with BCR627.

There is therefore a clear need to produce CRMs for As species to validate methods in general and to evaluate their accuracy, in particular. There is also a clear need for non-certified RMs for evaluation of the long-term reproducibility of methods (comparability of data over time), through the establishment of analytical quality-control charts, and for intercomparison studies for the evaluation of the comparability of data in space. However, information on the availability of non-certified RMs for As species (and for everything in general) is much more difficult to get, since producers of non-certified RMs are generally much smaller and not as well advertised as producers of CRMs. Information on RMs can be searched in the VIRM database for RMs available at the website of the Virtual Institute for Reference Materials (VIRM Asbl) (www.virm.net).

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