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Review

Arsenic and its speciation analysis using high-performance liquid chromatography and inductively coupled plasma mass spectrometry

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Abstract

It is known that arsenic has different toxicological properties dependent upon both its oxidation state for inorganic compounds, as well as the different toxicity levels exhibited for organic arsenic compounds. The field of arsenic speciation analysis has grown rapidly in recent years, especially with the utilization of high-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive and robust detector system. Complete characterization of arsenic compounds is necessary to understand intake, accumulation, transport, storage, detoxification and activation of this element in the natural environment and living systems. This review describes the essential background and toxicity of arsenic in the environment, and more importantly, some currently used chromatographic applications and sample handling procedures necessary to accurately detect and quantify arsenic in its various chemical forms. Applications and work using only HPLC–ICP-MS for arsenic speciation of environmental and biological samples are presented in this review.

Keywords: Reviews; Inductively coupled plasma mass spectrometry; Arsenic; Organoarsenic compounds

Contents

1. Introduction: the need for arsenic speciation analysis

In the past, analytical techniques using plasma spectrometry to determine arsenic levels have typically focused on

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the total level of arsenic, rather than the chemical form or the oxidation state. This approach is not adequate for today's analytical need; the actual toxicity levels for different arsenic compounds vary greatly. Inorganic arsenic is considered the most acutely toxic form [1]; arsenite (As^{III}) is more toxic than arsenate (As^{V}) [2,3]. The organic forms of have varying degrees of toxicity. Monomethylarsonic acid [MMA^{V}, $CH_3AsO(OH)_2$] and dimethylarsinic acid [DMA^{V}, $(CH_3)_2AsO(OH)$] exhibit a toxicity factor of one in four hundred that of the inorganic forms [4]. Furthermore, the inorganic compounds of arsenic have been classified as carcinogenic [5], and MMA^{V} and DMA^{V} have been identified as possible cancer promoters [6]. Trivalent arsenicals, including the organic compounds, have been reported to be more toxic than the pentavalent forms [7]. Monomethylarsonous acid [MMA^{III}, $(CH_3)As(OH)_2$] and dimethylarsinous acid [DMA^{III}, $(CH_3)_2AsOH$] are biotransformants of inorganic arsenic and have been reported to be very toxic [8–10]. On the other hand, arsenobetaine [AsB, $(CH_3)_3As^+CH_2COO^-$] is virtually non-toxic [11,12], and arsenocholine [AsC, $(CH_3)_3As^+CH_2CH_2O^-$] is believed to be non-toxic [13]. Many of these arsenic compounds are listed in Table 1. The chronic toxicity of arsenic compounds is currently not well understood and is actively being researched.

The legislative regulatory requirements instituted today should be supported by identification of the particular compound in which arsenic is present. This is part of the field known as elemental speciation. Elemental speciation is defined as the analytical activity of identifying and quantitating the actual chemical form, the chemical species, of an element and has been described in various reviews in the literature [14–19]. Speciation analysis of arsenic, therefore, usually requires the coupling of proper sample preparation with two analytical techniques: first, a technique to separate the chemical forms of arsenic, and second, a sensitive means of detection. Although some work in speciation analysis has been done using electrochemical detection of inorganic forms or arsenic [20,21] and optical emission spectroscopy (AES) for both organic and inorganic arsenic compounds [22–24], the inductively coupled plasma-mass spectrometer (ICP-MS) detector has been employed for almost two decades and provides the best level of sensitivity, an important feature considering the toxicity of some arsenic compounds. The ICP-MS detector, when coupled with high performance liquid chromatography (HPLC), offers the versatility of analysis for both organic and inorganic arsenic compounds. ICP-MS has become the favored detection technique for speciation analysis of arsenic [25].

Table 1
Some of the common arsenic compounds found in speciation analysis

Name	Abbreviation	Chemical formula
Inorganic compounds		
Arsenite (arsenous acid)	As^{III}	$As(OH)_3$
Arsenate (Arsenic acid)	As^{V}	$AsO(OH)_3$
Organic compounds		
Monomethylarsonous acid	MMA^{III}	$CH_3As(OH)_2$
Monomethylarsonic acid	MMA^{V}	$CH_3AsO(OH)_2$
Dimethylarsinous acid	DMA^{III}	$(CH_3)_2AsOH$
Dimethylarsinic acid	DMA^{V}	$(CH_3)_2AsO(OH)$
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2COO^-$
Arsenocholine	AsC	$(CH_3)_3As^+CH_2COO^-$
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$
Tetramethylarsonium ion	Me_4As^+	$(CH_3)_4As^+$
Arsenic-containing ribosides	Arseno sugars	Various sugar structures

1.1. Arsenic in the environment, human exposure and regulatory actions

Arsenic is introduced into the environment from a number of sources. The main sources for the aquatic environment is from geological sources, either from surface weathering or underground deposits. Human activities do also play a role. Arsenic compounds have been used in herbicides and pesticides for many years [26,27]; the run-off from agricultural activity has been a persistent problem. Lead arsenate was used both as an insecticide and herbicide; lead arsenite was used as an insecticide for many years before being replaced by more advanced organic compound insecticides. Industrial uses for arsenic have included its use in the manufacture of semiconductors and in the use as a wood preservative [26–28]. Arsenic is of interest because of the possible accumulation in food, which is the main source of human exposure. Water represents a secondary source of human exposure and is also a concern [29]. Many aryloarsenicals are used in animal feed additives and can find their way into the human diet [30]; the compound, 4-hydroxy-3-nitrophenylarsonic acid, is commonly used as a growth promoter in chicken feed.

Arsenic contamination of drinking water is also a concern and has been an important topic in the recent literature [7,29,31,32], as well as the focus of many world governments' health policies. Groundwater arsenic contamination in West Bengal and other regions of the Indian subcontinent have spurred research in the development of analytical techniques to monitor arsenic in its various chemical forms. The World Health Organization (WHO) level of arsenic in drinking water is 10 μg/L, and the current standard for drinking water in the USA is 50 μg/L, which was established in the 1940s by the US Public Health Service. The US standard is planned to be lowered to 10 μg/L by year 2006 which is a recent guidance issued by the US Environmental Protection Agency (USEPA). These arsenic limits refer to total arsenic, which makes a compelling need for regulation based on individual arsenic compounds and is further support for more speciation analysis.

1.2. The toxicology of arsenic

As was described earlier in the introduction, the chemical form and oxidation state of arsenic is very important with re-

gard to toxicity. Acute toxicity also is dependent upon other factors including physical state [gas, solution, or powder particle size], the rate of absorption into cells, the rate of elimination, the nature of the chemical substituents in the toxic compound and the general state of the patient. While it has been generally accepted in the past that methylation is the principal detoxification pathway, Vega et al. [33] have suggested that methylated metabolites may be partly responsible for the adverse effects associated with arsenic exposure. More recent thought has held that methylation, may in fact, be an activation step for arsenic with respect to chronic toxicity. Arsenic speciation becomes very important as an analysis tool used to increase the understanding of the chronic toxicity of arsenic species, including arsenic's mode in carcinogenicity, and in particular, the potential role of arsenic compounds in chromosomal alterations [29]. Arsenite has been shown to disrupt mitosis and induce apoptosis in human skin fibroblasts [34]. Also, it has been reported that inorganic arsenic can act as a transplacental carcinogen in mice [35]. The mechanisms and metabolic steps of chronic arsenic exposure are not well known and are currently being studied by many researchers today.

Studies in laboratory animals have demonstrated that the acute toxicity of arsenic is dependent upon its chemical form and its oxidation state. Again, chronic toxicity is not as well defined. Several studies of arsenic in animal and human cell lines would suggest that trivalent arsenic compounds are more acutely toxic than pentavalent forms. Because inorganic arsenite (As^{III}) is extensively metabolized in humans, chronic exposure to inorganic arsenic results in chronic exposure to methylated and dimethylated arsenic compounds. The most common acute toxic mode of this chemical element is the inactivation of enzyme systems; these enzymes serve as biological catalysts and are vital to life [36]. At high levels of arsenic, the enzymes responsible for generating cellular energy by means of the citric acid cycle can be adversely affected by arsenite. The inhibitory action is based on the inactivation of pyruvate dehydrogenase due to complexation with inorganic arsenite. The generation of adenosine-5-triphosphate (ATP) is prevented, causing damage or death to the exposed cells. It is believed that trivalent arsenic species, such as arsenite, can bind strongly to dithiols, as well as sulfhydryl groups, and other protein binding is possible. Such protein binding has implications in inhibited DNA repair, and would provide a possible mechanistic explanation for the observed increase in the incidence of carcinomas in the skin from arsenic exposure. Arsenate (As^{V}) at acute levels can also be disruptive as a competitor to phosphate. The prime example of arsenate's acute toxic mechanism is the suspension of oxidative phosphorylation. Oxidative phosphorylation is the process by which ATP is produced, while simultaneously, reduced nicotinamide adenine dinuclotide (NADH) is oxidized. This process is disrupted by arsenate when it forms an arsenate ester of ADP, which is unstable and quickly undergoes hydrolysis non-enzymatically. This hydrolysis process of the arsenate ester is known as arsenolysis [37], and the metabolic energy of the cell is inhibited causing cell damage or death.

Challenger and coworkers [38,39] reported that microorganisms grown in the presence of arsenite, MMA or DMA release trimethylarsine. A sequence of alternating reduction and methylation reactions was postulated for different mammals dosed with inorganic arsenic [39]. These reactions are catalyzed by enzymes and have been described in the literature [29,38–45]. There is, however, considerable variation in arsenic methylation products among different biological and animal species. In higher mammals, it is believed that arsenite (As^{III}) is first reduced to arsenate (As^{V}), which is methylated to MMA^{V}; MAA^{V} is reduced to MAA^{III}, which is methylated to DMA^{V}, and DMA^{V} is finally reduced to DMA^{III} These methylation reactions are catalyzed by enzymes and all the steps and mechanisms of these arsenic biotransformations have not been fully elucidated. Further arsenic speciation analysis is needed to gain a better grasp of the biological pathways and the forms of arsenic within a living system.

2. Inductively coupled plasma MS and interfacing HPLC to inductively coupled plasma MS

The topic of inductively coupled plasma mass spectrometry (ICP-MS) used in general speciation analysis has been reviewed in the literature extensively [14–19,46]. ICP-MS was developed by both the Houk and Gray research groups over 20 years ago [47,48]. ICP-MS offers several advantages for general speciation analysis detection over other more traditional detectors including: multielement and multiisotope detection, and more importantly, high sensitivity along with a wide linear dynamic range of detection [49]. The majority of the chemical elements have a dynamic linear range of 4–11 orders of magnitude [50]. Although arsenic is monoisotopic, ICP-MS offers an ideal detection system for arsenic speciation analysis owing to its high sensitivity.

In ICP-MS, the high efficiency of atomization and ion formation of the inductively coupled plasma (ICP) is coupled with the specific and sensitive detection capability offered by mass spectrometry. The complete description of the ICP need not be covered within this review; it has been described often in the literature [14–16,51]. The ICP is a high energy and "hard" ionization source; the plasma typically operates at temperatures of 5000–10 000 K at atmospheric pressure. Essentially, an aerosol of the sample is introduced into the plasma source where vaporization, atomization and ionization of the analyte occur nearly simultaneously. Elemental ions are passed on into a mass spectrometer.

Arsenic does have a spectral interference under certain conditions. Arsenic is monoisotopic and has a mass of 75 amu. Argon from the plasma gas and chlorine from the sample matrix may combine to form $^{40}Ar^{35}Cl$ which has the same nominal mass-to-charge ratio as arsenic. Thus, when monitoring the signal at m/z 75, the signal comes

from two sources, the arsenic and the argon chloride interference. A mathematical correction can be used to eliminate this interference. The portion of the signal generated by the argon chloride may be calculated and subtracted from the signal at *m*/*z* 75. Chlorine has two isotopes, ^{35}Cl and ^{37}Cl, so $^{40}Ar^{37}Cl$ should also form in the plasma at the isotopic ratio of chlorine 35 and 37. By monitoring *m*/*z* 77, the portion of the signal at *m*/*z* 75 generated from argon chloride may be subtracted allowing for the accurate determination of the arsenic signal. Certain plasma conditions may also be used to minimize the potential for this interference. It has been reported that introducing a reaction cell or collision cell prior to a quadrupole mass spectrometer will overcome the difficulty of polyatomic interactions [52]. In chromatographic systems, chloride interference can be eliminated by the controlled elution of chloride, either before or after the peaks associated with the arsenic species of interest. Ritsema et al. [53] reported a gradient elution anion-exchanged system using carbonate buffer where chloride eluted after the arsenic species.

Interfacing the ICP-MS detector and HPLC is relatively simple. The ICP-MS is designed for liquid sample matrices. Connecting an HPLC column to this nebulizer offers few problems. The typical 1 ml/min flow rate of an analytical HPLC column matches the usual flow rate of the commonly used pneumatic nebulizers used for ICP. Low flow rate (100 μl/min) nebulizers are also commercially available, so small bore and microbore HPLC can also be used with the ICP-MS detector.

The main problems of interfacing HPLC to ICP-MS concern the fundamental nature of the detector. One of the primary rules of HPLC is that the mobile phase used must be compatible with detection system. Sodium or potassium phosphate buffer mobile phase systems are often utilized in reversed-phase HPLC analysis when UV–vis detectors are used. This is because these phosphate buffers are UV transparent; however, these buffer mobile phases are not appropriate for the MS detector. Non-volatile buffer salts can collect on the lenses and skimmer cones resulting in signal drift and a high maintenance level for cleaning the inner surfaces of the MS detector. This dictates the use of volatile buffer systems or ones that have low residue after exiting the plasma of the ICP-MS system [54]. Ammonium salts of organic acids, as well as ammonium carbonate, are quite acceptable to the ICP-MS for low residues. Nitric acid mobile phases have also been reported for arsenic speciation analysis [55], however, arsenite (As^{V}) artifact peaks have been noted in arsenic compound seasoned columns exposed to nitric acid [54]. Therefore, oxidative nitric acid mobile phases would generally not be compatible with arsenic species stability. Also, the inductively coupled plasma has its own preferences for mobile phase content. HPLC techniques often use an organic modifier in the mobile phase; large volumes of organic solvent reaching the ICP result in an unstable plasma [56]. This problem can be minimized by desolvating the liquid aerosol before it reaches the ICP. A cooled spray chamber can be used to help condense a large portion of the solvent vapor and, thus, maintain plasma stability. Also, simple flow splitting after the HPLC column, or the use or a small bore or microbore column, can reduce the amount of organic solvent introduced into the detector. The organic modifier, itself, can play a role in plasma stability. Methanol is more widely used than acetonitrile for reversed-phase HPLC mobile phases, because it causes less plasma instability [56].

3. HPLC separation of arsenic compounds

HPLC has the advantage of performing separations of non-volatile species; thus it has greater versatility over gas chromatography, which often requires a derivatization of analytes before analysis. HPLC has the inherent characteristic of reproducibility of separation; the lack of this trait often plagues many capillary electrophoretic (CE) separations. Two of the earliest examples of HPLC being coupled to ICP-MS were by Thompson and Houk [57] and Dean et al. [58]. In the Thompson and Houk study [57], absolute detection limits of arsenic in the range of 0.1–0.2 ng were reported for their HPLC–ICP system. In arsenic speciation analysis, ion-exchange chromatography is the most extensively used, followed closely by the use of ion-pair chromatography. In an early work of arsenic speciation using HPLC–MS, Beauchemin et al. [59] studied both ion-exchange and reversed-phase ion-pairing chromatography. The Beauchemin study [59] also demonstrated low absolute detection limits for arsenic; the detection limits were reported to be between 50 and 300 pg. As described previously, these low detection limits and the ease of coupling most HPLC systems to the ICP-MS, makes ICP-MS such a successful detection method for arsenic speciation analysis. Micellar HPLC has also been used for some arsenic speciation analysis [60]. The other separation modes of chromatography (i.e. reversed-phase, size exclusion) are often used for other general elemental speciation analysis, but the majority of the work described in the literature utilizes ion-exchange or ion-pair chromatography for arsenic speciation analysis. Descriptions of the different chromatographic separation modes and specific examples in the literature will discussed in more detail.

3.1. Ion-exchange HPLC

Ion-exchange chromatography, by its basic design, is used for the separation of ions and easily ionized substances. Ion-exchange chromatography utilizes the mechanism of exchange equilibria between a stationary phase, which contain surface ions, and oppositely charged ions in the mobile phase. Ion-exchange HPLC many be used in either of two separation modes: anion or cation-exchange. The ionic strength of the solute, the pH of the mobile phase, the ionic strength and concentration of the buffer, and the temperature can all influence the separation and retention of analytes in

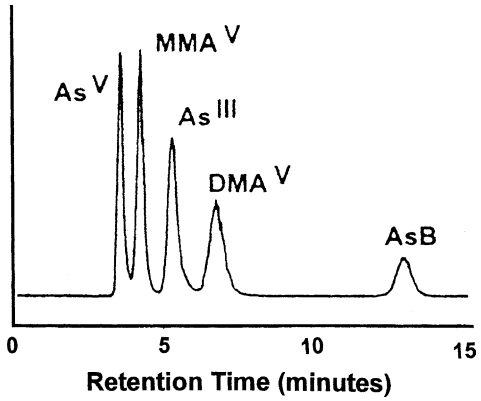

Fig. 1. Cation-exchange chromatogram showing the separation of arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), arsenite (As^{III}), dimethylarsinic acid (DMA^{V}), and arsenobetaine (AsB). The cation-exchange column was a Shodex Rspak NN-614, 150 mm × 6.0 mm i.d. The mobile phase was aqueous and 36 mM formic acid and 2 mM ammonium formate (pH 2.8). A flow rate of 0.8 ml min^{-1} was used. From ref. [45] with permission, copyright 2002, Elsevier Science.

ion-exchange HPLC, as do the standard variables such as flow rate and the introduction of organic modifiers into the mobile phase.

Both anion and cation-exchange chromatography have been used for arsenic speciation analysis. Anion-exchange is often used to separate arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) with minimal retention of arsenobetaine (AsB) and arsenocholine (AsC), while cation-exchange is often used to separate AsB, AsC, trimethylarsine oxide (TMAO) and tetramethylarsonium ion (Me_4As^{+}). Larsen et al. [61] published this type of separation with anion and cation-exchange columns when separating arsenic species from mushroom extracts. In an interesting exception, Suzuki et al. [44,45,62] developed a cation-exchange system that was capable of separating As^{V}, MMA^{V}, As^{III}, DMA^{V}, and AsB which used a formic acid/ammonium formate buffer system (Fig. 1).

Common buffer systems for ion-exchange chromatography used to separate the polar compounds of arsenic have included phosphate [63,64], carbonate [54,65], phthalic acid [66], tetramethylammonium hydroxide [67], and formate [62] buffers. As mentioned previously, sodium or potassium phosphate buffers are generally not desirable owing to the residue left upon the sampler and skimmer cones of the ICP-MS; however, ammonium carbonate leaves little residue. Ammonium carbonate buffered anion-exchange systems have shown little signal drift after prolonged use; B'Hymer and Caruso [54] reported no excessive residue on the sampler/skimmer cones after fifteen hour chromatographic run intervals. The 5000–10 000 K plasma leaves little other than ammonia, carbon dioxide and water and hence, ammonium carbonate is one of the preferred buffers for the ICP-MS. Phthalic, formic acid and tetramethylammonium hydroxide buffers would have the same advantage with the ICP-MS detector. Milstein et al. [73] reported a tris acetate [tris(hydroxymethyl)aminomethane acetate] buffer system to be superior to a phosphate buffered system studied for minimizing residue collection in the ICP-MS detector.

In a traditional anion-exchange system, Sakai and Yishi [69] and later Day et al. [70] used a mobile phase containing 2 mM sodium phosphate and 0.2 mM EDTA at pH 6 to separate As^{III}, As^{V}, MAA^{V} and DMA^{V} within a ten minute elution time. In the Day work [70], several tap water sample were analyzed. This isocratic system did elute some neutral arsenic species near the dead volume of the chromatograms. Detection limits of the four species were found to range from 0.4 to 0.8 μg L^{-1}.

Both isocratic and gradient ion-exchange chromatographic systems have been used to separate arsenic compounds. Isocratic systems generally dominate most reported separations, but gradient separations generally achieve peaks having higher plate number and better resolution of arsenic species in addition to reduction of retention times compared to isocratic chromatography. Separation of AsB, As^{III}, As^{V}, MMA^{V} and DMA^{V} was reported by Lintschinger et al. [67] using gradient elution with 2 mM tetramethylammonium hydroxide and 10 mM ammonium carbonate binary mobile phase. The five species were baseline resolved, within a ten minute run time, in the analysis of urine samples (Fig. 2). In another anion-exchange system, B'Hymer and Caruso [54] separated AsC, AsB, As^{III}, DMA^{V}, MMA^{V} and As^{V} with a gradient anion-exchange chromatographic procedure using a Hamilton PRP-X100 column and an ammonium carbonate buffer, pH 8.5; the gradient concentration range was from 12.5 to 50 mM (Fig. 3). Isocratic conditions based on this column and chromatographic system were employed to quantify As^{III}, As^{V} and DMA^{V} levels in extracts of

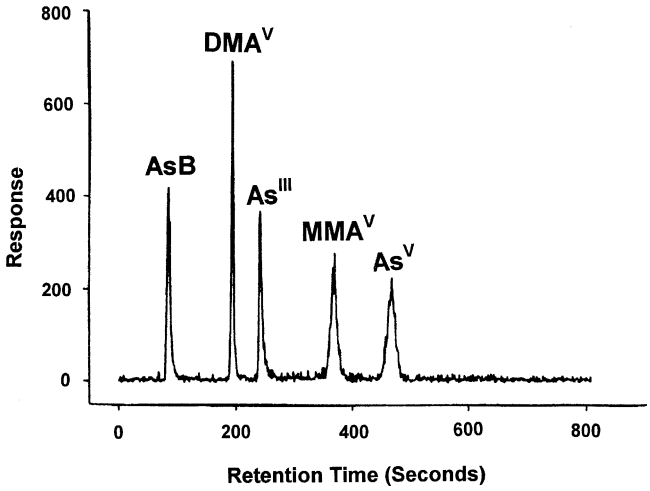

Fig. 2. Chromatogram of arsenic species using a gradient ion-exchange system separating arsenobetaine (AsB), dimethylarsinic acid (DMA^{V}), arsenite (As^{III}), monomethylarsonic acid (MMA^{V}), and arsenate (As^{V}). The column was a Dionex IonPac AS12 4 mm i.d., 46124. Mobile phase A was aqueous 2 mM tetramethylammonium hydroxide (TMAOH). Mobile phase B was aqueous 10 mM ammonium carbonate (pH 10.0). Flow rate was 1.5 ml min^{-1} with the following gradient program: 0–0.5 min, 100% A; 0.5–8 min 100% A to 100% B and 8–15 min 100% B. From ref. [67] with permission, copyright 1998, Springer-Verlag.

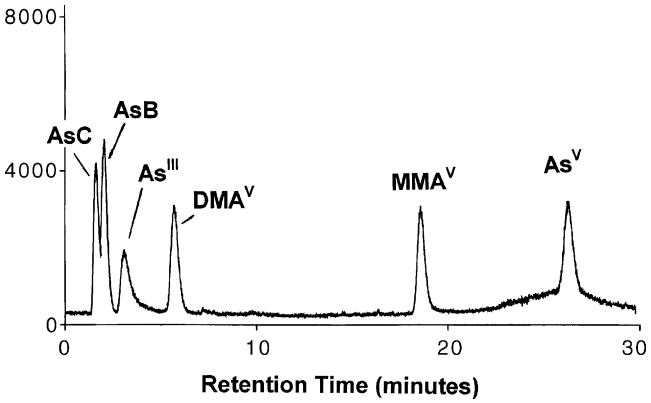

Fig. 3. Chromatogram of arsenic species using a gradient ion-exchange system separating, arsenocholine (AsC), arsenobetaine (AsB) dimethylarsinic acid (DMA^V), arsenite (As^{III}), monomethylarsonic acid (MMA^V), and arsenate (As^V). The column was a Hamilton PRP-X100 anion-exchange 150 mm × 4.1 i.d. Mobile phase A was aqueous 12.5 mM ammonium carbonate (pH 8.5). Mobile phase B was aqueous 50 mM ammonium carbonate (pH 8.5). The flow rate was 0.8 ml min^{-1} with the following gradient program: 0–8 min 100% A, 8–20 min 100% A to 100% B, 20–30 min 100% B. From the authors' collection, see conditions in ref. [54].

freeze-dried apples [54]. Modifications to the gradient ramp reported in the B'Hymer and Caruso study [54] were later used to quantify arsenic compounds in lobster tissue [71]. Sloth et al. [72] used a gradient cation-exchange system to determine the organoarsenic species in marine samples.

Multi-mode or mixed mode ion-exchange chromatography has also been used in arsenic speciation analysis. Milstein et al. [73] used a multi-mode ion-exchange chromatographic system to separate As^{III}, As^V, MMA^V, DMA^V, AsB, AsC, and the results are shown in Fig. 4. An ammonium carbonate buffer system was used (between 10 and 50 mM)

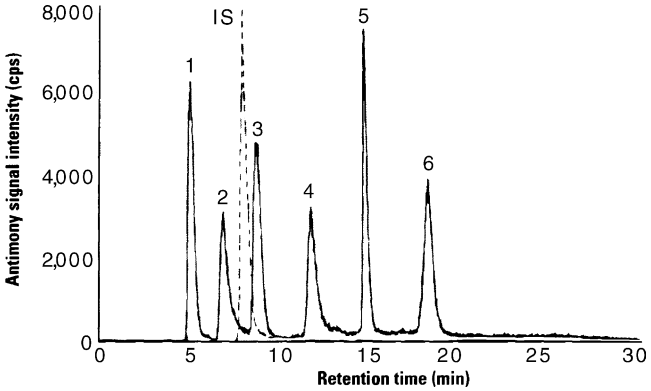

Fig. 4. Chromatogram of arsenic species using a multi-mode (mixed) ion-exchange gradient. Peaks: 1, AsB; 2, As^{III}; 3, DMA^V; 4, AsC; 5, MMA^V; 6, As^V; "IS" is the internal standard (potassium hexahydroxy antimonate). A Hamilton PRP-X200 column (150 mm × 4.1 mm i.d.) was connected in series with a Hamilton PRP-X100 (250 mm × 4.1 mm i.d.) using a buffer gradient (10–50 mM) of ammonium carbonate at pH 9.0 and a flow rate of 1.0 ml/min. Mobile phases A and B were water–methanol (94:6, v/v). The gradient program was 0–7 min, 100% A to 100% B and hold at 100% B. From Ref. [73] with permission from Environmental Health Perspectives.

with two columns, a cation and an anion-exchange column connected in series. Baseline resolution of the arsenic species was obtained with this system. Other multi-mode ion-exchange systems have been successfully used in arsenic speciation analysis. In an isocratic multi-mode system using ammonium nitrate and nitric acid buffer, As^{III}, As^V, MMA^V, DMA^V and AsB were separated in standard water solutions, and this methodology was applied to field water samples [74].

3.2. Ion-pair HPLC

Simple reversed-phase HPLC uses mobile phases that are aqueous solutions which may contain a portion of organic modifiers. The separation of analytes is performed using stationary phases that have a surface less polar than the mobile phase. In reversed-phase ion-pair chromatography, a counterion is added to the mobile phase, and a secondary chemical equilibrium of the ion-pair formation is used to control retention and selectivity. The important advantage of ion-pair HPLC is that it facilitates the separation of ionic species as well as uncharged molecular species. Commonly used ion-pair reagents are long-chain alkyl ions, such as alkylsufonates with chain lengths usually of C-5 and greater or tetralkylammonium salts. Ion-pair reagent concentrations in the mobile phase are usually kept low (approximately 0.02 M and less). Elution and separation are achieved using aqueous solutions with an organic modifier, usually methanol when using the ICP-MS detector for arsenic speciation analysis. Separation of analytes in ion-pair chromatography is influenced by several variables including hydrophobicity of the counter ion, the concentration of the ion-pair reagent, buffer concentration, the pH and ionic strength of the mobile phase, and properties of the stationary phase.

Arsenic speciation was demonstrated by B'Hymer et al. [75] using isocratic ion-pair HPLC with modified conditions similar to those published by Le et al. [76]. A small bore C18 column was used with a 100% aqueous mobile phase containing 25 mM citric acid and 10 mM pentane sulfonic acid sodium salt at pH 2.4. MMA^V, DMA^V, AsB and AsC were all separated within a half hour chromatographic run time. Various nebulizers were used in the B'Hymer study [75], and detection limits as low as 1 ug/L (ppb) were obtained; all peaks exhibited good symmetry and shape. These HPLC conditions were later evaluated by Ackley et al. [77] for the separation of arsenic compounds extracted from certified reference material (DORM-2 and spiny dogfish muscle). Failure to match the sample injection matrix with the aqueous mobile phase resulted in poor chromatographic performance in the separation of arsenic compounds [77].

Gradient ion-pair liquid chromatography is not commonly used with arsenic speciation when using the ICP-MS detector. Signal drift is likely when substantially changing the organic content of the mobile phase. B'Hymer and Caruso [54] demonstrated an ion-pair system using a shallow gradient. Standards solutions containing As^V, As^{III}, MMA^V,

DMA^V, AsB and AsC were chromatographed using a mobile phase consisting of 40 mM citric acid and 10 mM hexane sulfonic acid sodium salt. Aqueous–organic content varied from 98:2 or 96:4 (v/v) initial mobile phase A, over a 10% organic content increase in mobile phase B [either 88:12 (v/v) or 86:14 (v/v) water/methanol] for a 30 min gradient period. Flow rates were fixed, and it was found that 0.8 ml/min yielded the best separation of the arsenic compounds. None of the conditions examined in this study were able to fully resolve the As^{III} and MMA^V peaks. Baseline drift from the ICP-MS system was noticeable, but acceptable for integration of the peaks in the chromatogram. The inductively coupled plasma remained stable with methanol content at 14% (v/v) at a flow rate of up to 1.0 ml/min.

In what may be one new trend, fast elution of arsenic species has been demonstrated using ion-pair HPLC. Wrobel et al. [78] reported the elution of As^{III}, As^V, MMA^V, DMA^V and AsB in roughly a 4 min time span using a heptanesulfonic acid and citric acid buffer system. Arsenocholine, AsC, was not included in this separation analysis. This study reported reasonable day-to-day reproducibility and reported the use of actual urine samples. Although this chromatographic system offered distinct advantages in short run times, some loss of resolution between As^V and MMA^V was noted when actual urine samples fortified with analytes were chromatographed [78]. This raised the point as to whether, in general, other fast chromatographic methods, having minimal retention analytes, will be applicable to more complicated sample matrices. Resolution of analytes tend to suffer more in fast chromatographic systems as compared to traditional chromatographic systems with longer retention of analytes, particularly when using sample matrices that affect chromatographic separation and performance.

3.3. Micellar HPLC

Micellar liquid chromatography is another variation of reversed-phase HPLC and has been used for arsenic speciation analysis. In micellar chromatography, a relatively high concentration of a surfactant is used as counterions and the formation of "micelles" occurs. Although there are few applications of micellar HPLC with the ICP-MS detector to date, one interesting example was used in arsenic speciation by Ding et al. [60]. In this study, the separation of As^{III}, As^V, MMA^V and DMA^V was accomplished using cetyltrimethylammonium bromide at 0.05 M to form the micelles in a 90/10 (v/v) aqueous/propanol mobile phase containing 0.02 M borate buffer. This study included the analysis of urine samples, which has direct applications to arsenic speciation needed for current analytical problems.

4. Sample preparation procedures

A critical requirement for obtaining accurate arsenic speciation information is in maintaining the concentration and chemical forms of the original species through the sample extraction and preparation. The correct sampling and sample preparation procedure is essential to obtain accurate speciation analysis data. Maintaining the chemical form of arsenic during sampling and sample preparation is undoubtedly the most fundamental challenge of analysis. Contamination, representativeness of the sample, the possibility of precipitate and wall effects from the sample container are additional concerns in speciation analysis. Typically, mild extraction methods need to be employed to reduce the risk of chemical species alterations. With arsenic analysis, oxidation of the species during sample handling and preparation can be a problem. As^{III} has been known to oxidize to As^V during some sample preparation and storage procedures [63,79–82], Also, spiked sample validation studies are required to verify that chemical species are not altered during all the stages of speciation analysis.

The case studies of arsenic species stability in the literature are numerous. For example, in water analysis, As^{III} and As^V are the major arsenic components. The procedures used for sample preparation and analysis have been shown to result in the oxidation of As^{III} to As^V [79–82]. Difficulties also arise in iron-rich natural water. Soluble arsenic species can form insoluble iron precipitates in water with high iron concentrations. EDTA has been used to prevent the formation of arsenic/iron precipitates [70,83]. Ascorbic acid and hydrochloric acid have been used and studied as preservatives of As^{III} and As^V in natural waters [84]. Chelation agents have also been used. The pentavalent methyl arsenicals are generally more stable than the trivalent inorganic and organic forms. Palacios et al. [85] found MMA^V, DMA^V and AsB to be relatively stable in urine for extended periods; Larsen et al. [79] also reported that concentrations of those arsenic species to be relatively constant in aqueous solutions. Caruso et al. [63] reported reasonable recovery of known spike levels of MAA^V and DMA^V from extracts of freeze-dried apples, but found some evidence of oxidation of As^{III} to As^V.

The general process of sampling consists of collection, storage, pretreatment or treatment and finally, a concentration or extraction step. Each of these steps may alter the results of arsenic speciation analysis, and they must be considered carefully when designing an appropriate procedure for sample analysis.

4.1. Sample collection, storage, and treatment

Generally, the initial step is collection of the actual sample and species preservation must be considered. Sample containers must be considered for their possible wall effects. Material may be adsorbed or lost by choosing the wrong container material. Nitric acid is commonly used to wash/rinse many types of containers, but any remaining acid would cause oxidation of arsenic species in a sample solution stored in such a container. That brings to a point the need to consider pre-treatment options. Urine samples are

often pre-treated or preserved by the addition of nitric acid [86]. This procedure is suitable for the measurement of total arsenic, but would cause oxidation of the chemical species and is not appropriate for speciation analysis. Non-oxidizing acids should be used in speciation analysis. In the case of the ICP-MS detector, hydrochloric acid may also be undesirable due to the addition of chloride as an interferent for arsenic. Sample storage could also present problems; long term freezing of samples is generally acceptable, although some exceptions have been reported. AsB in sample extracts stored at 4 °C for nine months was found to decompose to trimethylarsine oxide and two other species [87]. Deep-freezing samples will generally minimize any bacterial or enzyme degradation or loss from volatility. Poorly sealed sample containers may add oxygen for oxidation or loss of sample if the compounds are volatile. Bacterial degradation of the sample should be avoided. Bacteria obviously can convert inorganic arsenic to methylated forms and steps should be taken to preserve the original samples. Sample cleanup from a biological or complicated matrix can present problems. Ultimately, a stability study using samples spiked with known arsenic species is necessary to validate a sample storage and treatment procedure.

4.2. Sample concentration and extraction

Sample concentration and/or extraction procedures have consisted of traditional approaches such as solid–liquid or liquid–liquid extraction, solid phase extraction (SPE) and solid-phase microextraction (SPME). Solid sample preparation generally includes milling, grinding, freeze-drying or sieving followed by some form of extraction. Leaching (solid–liquid extraction) or Soxhlet extraction is commonly practiced. Enhanced techniques such as pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) have all been used in arsenic analysis.

4.2.1. Traditional techniques

Extraction by leaching solid or biological samples is very common. Extraction is most often performed using methanol–water solvent systems or occasionally acetonitrile–water [63,77]. Agitation for the extraction can be simple physical movement or by sonication. Sequential extractions are not uncommon. Treatment initially with enzymes have also been used with some food stuffs; freeze-dried apple samples were so treated with α-amylase [54,63]. The α-amylase was found to assist in breaking up the cellulose in freeze-dried apple samples and general improved extraction yields of arsenic species; this treatment was followed by extraction with acetonitrile–water [54,63]. A trypsin digest procedure has been reported on fish samples; AsB species was found not to decompose with this process [88]. Sequential extraction was used to extract arsenic species from fish tissue by McKiernan et al. [89]; acetone was used to remove fats and lipids from the mixture and then water–methanol 150:150, (v/v) was used to extract the arsenic species. Defatting by leaching with acetone has been practiced to study seafood previously [90]. Simple hot water extraction was used to extract arsenic compounds from human finger nails [91]. Other extraction procedures cited in the literature for arsenic speciation analysis include basic Soxhlet extraction [92] and solid-phase extraction [93].

4.2.2. Enhanced techniques

Thermal and microwave heating has been used in arsenic speciation analysis. Since the preservation of the organoarsenic moiety is the prerequisite of a successful extraction procedure prior to speciation analysis, careful optimization of microwave conditions have been applied to samples and reported in the literature [77,94,95]. The use of the microwave for extraction has become very popular for both trace metal analysis and for elemental speciation [96]. In general, a focused low power microwave field can be preferred over high temperature or traditional acid extraction techniques for the removal of organometallic species from a sample matrix as carbon–metal bonds are more likely to remain intact. The directed energy of the microwave can be controlled absolutely using the programming options of the current instruments commercially available. Closed sample vials are exposed to microwaves with controlled power, time, temperature and/or pressure. MAE was used to remove arsenic species from fish [77], and MAE was used to study As^{III} and As^{V} from plant materials [95]. MAE was demonstrated to give the highest recovery of total arsenic extracted from lobster tissue when compared to other extraction techniques [71], and MAE was used to extract arsenic species from marine microalgae [97].

Another enhanced extraction technique is pressurized liquid extraction or PLE (the Dionex trade name is ASE, for accelerated solvent extraction). It is an automated extraction techniques rapidly gaining acceptance owing to the availability of commercial extraction systems. The general process consists of an extraction solvent pumped into a sample cell and heated for a prescribed time. Compressed gas is used to push the extraction solvent through a filter, or filter and bed, into a collection vial. Most instrumental systems can be programmed at various temperatures and heating/static times for the solvent within the sample cell. Gallagher et al. [98] used PLE (the ASE system) to extract sea kelp, and the same system was also used by Vela et al. [99] to extract arsenic species from carrots. A comprehensive evaluation and study using this technique to extract arsenic compounds from plants was done by Schmidt et al. [100].

SFE has been used to extract arsenic species from different sample matrices. SFE is based upon the physical principle that when a substance is heated above its critical pressure and temperature, it may exist in a supercritical fluid state. Solubilization of analyte compounds may then be more easily performed from a sample matrix. Lobster tissue was extracted using water SFE, as well as more conventional extraction

techniques, and analyzed for different arsenic species by HPLC–ICP-MS [71]. Again, this is a technique by which conditions can be optimized by the current instrumentation to perform controlled and, hopefully, mild extraction conditions which preserve the original arsenic species found in the sample. Finally, it must be stressed that with all sample treatments and procedures, a demonstration by a recovery study of known arsenic spikes must be conducted to verify the accuracy of any procedure used in arsenic speciation analysis.

5. Further applications of arsenic speciation analysis

Only a few specific applications of arsenic speciation have been described in this review. Applications to specific samples include environmental samples, biological samples, and clinical samples from human beings. Water and soil represent the two most widely monitored samples from the environment. Generally inorganic forms of arsenic have been present in these matrices. Biological samples have included food samples, plants, algae and animal tissues. The widest varieties of arsenic species have been found in these sample matrices. Monitoring of foodstuffs is very important with respect to the human diet. Although marine animals typical have high levels of arsenic, it is generally in the form of the non-toxic arsenobetaine. Plant material food stuffs can have various arsenic levels dependent upon the type of plant. For example, the level of inorganic arsenic species quantified in apple extracts were reported in the μg/kg (ppb) range [54], allowing the conclusion that apples are of little concern as a source of arsenic in the human diet. Rice, however, was reported to have a high level of arsenic [101,102]. Finally, clinical samples monitoring humans have included bile, blood, and blood cells [44,45,62], urine [44,60,64–67,73,78,103–105], fingernails and hair [91] with the emphasis on specific arsenic species proposed as biomarkers for human exposure to arsenic. Urine is commonly used for arsenic exposure assessment, but total arsenic concentration in urine is positively related to the frequency of fish and seafood consumption in the human diet [106]. This makes arsenic speciation analysis more important for the monitoring of specific toxic arsenic biomarkers, not low risk dietary species such as arsenobetaine, to evaluate serious arsenic exposure. Table 2 gives some additional citations regarding applications of arsenic speciation, although it is obviously not comprehensive nor fully inclusive of all the publications available in this rapidly growing area of analysis. The table is organized by the sample matrix analyzed, the type of chromatographic analysis used (i.e., ion-exchange, reversed-phase, ion-pair, or micellar HPLC), and the arsenic species either separated or quantitated in that specific reference. Hopefully, this information will help those interested in reading more about arsenic speciation.

Table 2
Applications of HPLC with ICP-MS for arsenic seciation

Sample	Chromatographic system	Mobile phase composition	Arsenic species	Ref.
Apples	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, DMA^{V}	[54]
	Anion-exchange, isocratic	Aqueous, sodium phosphate and nitrate buffer	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[63]
Body fluids, blood, etc.	Cation-exchange, isocratic	Aqueous, formic acid and ammonium formate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[44,45,62]
Carrots	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[99]
Chicken tissue	Reversed-phase, isocratic	Methanol–water (5:95, v/v), phosphoric acid	4-Hydroxy-3-nitrophenyl-arsonic acid (poltry growth promotor)	[107]
Club soda	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[65]
Fish				
Various Fish	Anion-exchange, gradient	Aqueous, 1–50 mM potassium sulfate	As^{III}, As^{V}, AsB, DMA^{V}	[88]
Various Fish and DORM-2	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[89]
DORM-1	Anion-exchange, isocratic	Methanol–water (5:95, v/v), sodium phosphate buffer	As^{III}, As^{V}, AsB, MMA^{V}, DMA^{V}	[59]
	Ion-pair, both anionic and cationic pair, isocratic	(1) Anion-pair, Methanol–water (5:95, v/v), tetrabutylammonium hydroxide (2) Cationic-pair, acetic acid–methanol–water (2.5:5:92.5 v/v/v), sodium dodecylsulphate	As^{III}, As^{V}, AsB, MMA^{V}, DMA^{V}	[59]

Table 2 (*Continued*)

Sample	Chromatographic system	Mobile phase composition	Arsenic species	Ref.
DORM-2	Ion-pair, isocratic	Aqueous, citric acid buffer and hexane sulfonic acid sodium salt	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[78]
DORM-2, ocean whitefish, black tip shark, steel-head salmon	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[77]
	Ion-pairing, isocratic	Aqueous, citric acid buffer and pentane sulfonate sodium salt.	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC (arsenobetaine detected as primary component of all fish tested)	[77]
DORM-2, tuna, TORT-1 Lobster	Cation-exchange, gradient	Methanol–water (3:97,v/v), 0.5 to 20 mM pyridine/formic acid	MMA^{V}, DMA^{V}, AsB, TMAO and others	[108]
Lobster	Anion-exchange, gradient	Aqueous, 15 to 50 mM ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[71]
Marine micro-algae	Ion-exchnge, isocratic	(1) Anion-exchange, aqueous, amonium carbonate (2) Cation-exchange, aqueous, pyridine–formic acid	As^{V}, MMA^{V}, DMA^{V}	[97]
Mushrooms	Ion-exchange, isocratic	(1) Anion-exchange, Methanol–water (3:97,v/v), 45 mM ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[61]
		(2) Cation-exchange, methanol–water (3:97,v/v), 5 mM pyidine–formic acid	AsB, AsC, TMAO	
Plants, various terrestrial	Ion-exchange, isocratic	(1) Anion-exchange, aqueous, 20 mM ammonium phosphate	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[95]
		(2) Cation-exchange, aqueous, 20 mM pyridine-formic acid	As^{III}, AsB, AsC	
Plants, various	Anion-exchange, gradient	Nitric acid, 0.4–50 mM with 0.05 mM benzene-1,2-disulfonic acid	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB Note: AsC, TMAO and TMA not resolved in this system	[100,109]
Rice	Ion-exchange, isocratic	(1) Anion-exchange, aqueous, 10 mM phosphate buffer	As^{V}, MMA^{V}, DMA^{V}	[98]
		(2) Cation-exchange, aqueous, 4 mM pyridine–formic acid	As^{III}, AsB	
Standards	Anion-exchange, gradient	Aqueous, 12.5 to 50 mM ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[54]
	Ion-pair, gradient	2:12 and 4:14 (v/v) methanol–water, citric acid buffer and hexanesulfonic acid	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[54]
	Ion-pair, isocratic	Aqueous, citric acid buffer and pentane sulfonic acid sodium salt	MMA^{V}, DMA^{V}, AsB, AsC	[75]
Soil extracts	Anion-exchange, gradient	Nitric acid, 0.4 mM to 50 mM with 0.05 mM benzene-1,2-disulfonic acid	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB Note: AsC, TMAO and TMA not resolved in this system	[109]
	Anion-exchange, isocratic	Aqueous, sodium hydroxide	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[110]
Urine	Anion-exchange, isocratic	Aqueous, phosphate buffer	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[64]
	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[65]
	Anion-exchange, isocratic	Aqueous, phthalic acid buffer	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[66]

Table 2 (*Continued*)

Sample	Chromatographic system	Mobile phase composition	Arsenic species	Ref.
Urine, continued	Anion-exchange, gradient (binary)	Aqueous, 2mM tetramethylammonium hydroxide to 10 mM ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[67]
	Micellar, isocratic	Propanol-water (10:90, v/v), borate buffer with cetyltrimethylammonium bromide	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[60]
	Anion-exchange, isocratic	Aqueous, ammonium phosphate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[103]
	Ion-pair, isocratic	Aqueous, citric acid buffer and hexane sulfonic acid sodium salt	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB	[78]
	Anion-exchange, isocratic	Aqueous, citric acid and nitric acid	As^{III}, As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, DMA^{V}, AsB, AsC	[45,104]
	Anion-exchange, isocratic	Aqueous, 15 mM tartaric acid	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[105]
	Ion-pair, isocratic	Aqueous, 10 mM hexanesulfonate–1 mM tetraethylammonium hydroxide	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[111]
	Ion-exchange,multi-mode, gradient	Methanol–water (6:94, v/v), 10 to 50 mM ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[73]
Water	Anion-exchange, gradient	(1) Aqueous, phosphate buffer (2) Aqueous, tris buffer	As^{III}, As^{V}, MMA^{V}, DMA^{V} As^{III}, As^{V}, MMA^{V}, DMA^{V}	[68]
	Anion-exchange, isocratic	Aqueous, sodium phosphate and EDTA	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[70]
Water, spring	Ion-pair, isocratic	Aqueous, tertabutylammonium phosphate	As^{III}, As^{V}, MMA^{V}, DMA^{V}, AsB, AsC	[112]
Wine	Anion-exchange, isocratic	Aqueous, ammonium carbonate	As^{III}, As^{V}, MMA^{V}, DMA^{V}	[65]
	Ion-pair, isocratic	Aqueous, malonic acid with tertabutylammonium hydroxide	As^{III}, As^{V}, MMA^{V}, DMA^{V} plus 4-hydroxy phenyarsonic acid (internal standard)	[113]

6. Conclusions and future trends

Arsenic speciation analysis represents a field that will continue to grow in importance in the coming years. ICP-MS is the detection method of choice for interfacing with HPLC; it represents an effective hyphenated technique to perform arsenic speciation analysis. In addition, HPLC offers a rugged and versatile separation technique. Microbore HPLC columns will probably grow in use with the ICP-MS detector; lower volumes of mobile phase containing organic modifier will lend itself to a more stable plasma. Hydride generation (HG), a post-column derivatization process, is another technique. Although hydride generation was not discussed in detail in this manuscript, it generally has the advantage of better sensitivity and lower detection limits from a higher analyte transport efficiency into the ICP-MS detector. Nakazato et al. [114] used HG to determine As^{III}, As^{V} and MMA^{V} in seawater samples and Yehl et al. [115] used HG to determine arsenic species from soil extracts. However, HG consists of a more complicated interface, which seems to make it a less frequently used technique in arsenic speciation analysis. The direct connection of an HPLC system through a nebulizer/spray chamber arrangement to the ICP-MS system will likely dominate the field of arsenic speciation analysis.

One of the most important technical considerations in arsenic speciation is in sample preparation. Extraction of the arsenic compounds while preserving the original compound and arsenic oxidation state is a prime concern. Some of this was discussed briefly in this review; procedures using chelation and the use of antioxidants were mentioned. The enhanced techniques, such as microwave assisted extraction and accelerated solvent extraction, have made significant improvements in providing programmable and mild extraction conditions which can be optimized by careful study. Many sampling schemes will need to be improved over the coming years to obtain more accurate arsenic speciation analysis.

Finally, further characterization of arsenic compounds in various samples is an area of some challenge. Arsenosurgars are being characterized often and unknown arsenic species

are being found and studied with greater frequency. Parallel use of the electrospray MS along side the more sensitive ICP-MS is a rapidly growing trend to gain structural information or to help identify arsenic compounds. Also, the increasing availability of reference compounds and certified reference materials containing specific arsenic species will greatly assist the study of arsenic and speciation analysis.

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