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Review

As, Hg, I, Sb, Se and Sn speciation in body fluids and biological tissues using hyphenated-ICP-MS techniques: A review

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ABSTRACT

The present review deals with As, Hg, I, Sb, Se, and Sn speciation in biological tissues and body fluids carried out in the last ten years. The focus is inductively coupled plasma mass spectrometry (ICP-MS) as a powerful analytical tool for elemental speciation analysis. Methods based mainly on liquid chromatography–inductively coupled plasma mass spectrometry (LC–ICP-MS), gas chromatography–inductively coupled plasma mass spectrometry (GC–ICP-MS), capillary electrophoresis–inductively coupled plasma mass spectrometry (CE–ICP-MS) are highlighted. Applications of other hyphenated techniques are also included, but in lesser extent. Relevant applications of methodologies used for As, Hg, I, Se, Sb, and Sn speciation in biological tissues and body fluids are cited.

Contents

Abbreviations: AAS, atomic absorption spectrometry; AES, atomic emission spectrometry; AF, affinity chromatography; AFS, atomic fluorescence spectrometry; AsB, arsenobetain; AsC, arsenocholine; CC, collision cell; CE, capillary electrophoresis; DBT, dibutyltin; DMA(V), dimethylarsinic acid; DMA(III), dimethylarsonic acid; DMAA, dimethylarsinoylacetate; DMAE, dimethylarsinoylethanol; DMDSe, dimethyldiselenide; DMPS, sodium 2,3-dimercapto-1-propane-sulfonate; DMSe, dimethylselenide; DMT, dimethyltin; DPhT, diphenyltin; DRC, dynamic reaction cell; EDTA, ethylenediaminetetraacetic acid; EI-MS, electron-ionization mass spectrometry; ESI, electrospray ionization; EtHg, ethylmercury; FTICR, Fourier-transform ion cyclotron resonance; GC, gas chromatography; GE, gel eletrophoresis; GPx, glutathione peroxidase; HG, hydride generation; IC, ion chromatography; ICP, inductively coupled plasma; ID, isotope dilution; LC, liquid chromatography; LA, laser ablation; LOD, limit of detection; LOQ, limit of quantification; LT, low termperature; MALDI, matrix assisted desorption ionization; MBT, monobutyltin; MAE, microwave assisted extraction; MC, multicollector; MeHg, methylmercury; MMA(V), monomethyl arsenic; MMA(III), monomethylarsonic acid; MIP, microwave induced plasma; MMT, monomethyltin; MPhT, monophenyltin; MS, mass spectrometry; NMR, nuclear magnetic ressonance; OTs, organotin compounds; PhTs, phenyltins; QMS, quadrupole mass spectrometry; RP, reverse phase; SeCys, selenocysteine; SeAlb, selenoalbumin; SeMet, selenomethionine; SelP, selenoprotein P; SeProt, selenoprotein; SEC, size exclusion chromatography; SF, sector field; SPE, solid phase extraction; SPME, solid-phase microextraction; SSID, species specific isotope dilution; TBT, tributhyltin; TCHT, tricyclohexyltin; TMAH, tetramethylammonium hydroxide; TMAsO, tetramethylarsonic acid; TMAs, tetramethylarsenic; TMAO, trimethylarsine oxide; TeMA, tetramethylarsonium; TMSe, trimethylselenium; TMT, trimethyltin; $TMSbCl_2$, trimethylantimony dichloride; TOF, time of flight; TPhT, triphenyltin; USN, ultrasonic nebulization; VG, vapor generation.

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1. Introduction

The development of analytical strategies to obtain qualitative and quantitative information on elements, element species, their interactions, transformations and functions in biological systems is of increasing importance. This information is important for understanding toxicology and metabolic pathways of toxic and essential elements relevant to health/disease.

Speciation analysis has become increasingly important in making risk assessments of toxic elements since toxicity and bioavailability depend on the chemical form of the element. The general prerequisite for a meaningful speciation method is the ability to discriminate among the various chemical forms of the same element and the quantification of each of the species. Effective separation methods for different species of the element of interest, and suitable detectors to quantify the isolated species, are indispensable to meet this requirement. Species information of the investigated sample needs to be preserved through all the steps of the entire analytical procedure. Therefore, a key prerequisite for obtaining reliable species information is to maintain the concentration of the original element species prior to separation and quantification [1].

Most speciation methods have employed chromatographic techniques, being LC coupled to ICP-MS the combination of common use. ICP-MS offers many advantages, including high elemental selectivity, large linear range, and relatively low LOD. The use of ICP-CC-QMS or ICP-DRC-MS [2] or ICP-SFMS [3] decreases the background signal caused by molecular ion interferences by separating the analyte signal from that of the molecular ion. However, no information about the analyte species can be drawn by ICP-MS because complete ionization of the molecules in the plasma does not retain any molecular information.

LC, GC and CE can be combined with ICP-MS for the determination of different elemental species. However, species information can only be obtained by comparison with standards of each species. Molecular occurrence of the element of interest does not influence the response significantly and ICP-MS shows only limited matrix influence, which results in species-independent response. Hence, only one elemental species can be used for quantification purposes for a series of different species of a given element. This becomes very useful when the analyte is an unknown species or a species of which no pure standard is available. However, the LOD for different species can be slightly different because the peak width of the eluting species may vary when LC, GC or CE are used for species separation.

The main objective of this review is to illustrate speciation analysis of As, Hg, I, Sb, Se and Sn in body fluids and biological tissues carried out during the past decade. These elements, as others, play an important role (as essential or toxic element) in biological systems and have been studied in speciation analysis. This review was as comprehensive as possible and not all works published in the last decade could be included, because they could not be accessed and/or were not published in English.

2. Hyphenated techniques

Hyphenation of LC, GC, CE, as separation techniques, with ICP-MS opened new possibilities for element speciation analysis in a variety of samples.

Liquid chromatography is relatively easy to be coupled to the plasma torch of the ICP and major modifications of the standard introduction system of the ICP-MS instrument are not necessary. If the flow rate of the mobile phase is compatible with the nebulizer solution uptake, the column outlet can be connected directly to the pneumatic nebulizer. To do so, there exist nebulizers that can operate in a wide range of solution uptake (from $nL\,min^{-1}$ to $mL\,min^{-1}$), making possible the interface with standard LC, μ-LC or nano-LC systems [4–7]. Instability of the analyte signal, decreased sensitivity and worsening in chromatographic resolution are the main difficulties observed when low flow LC systems are employed [6–13]. In general, the main problems observed in LC–ICP-MS are related to the composition of the mobile phase, whose concentration of salts and organic solvent must be as low as possible for mobile phase introduction into the plasma.

The LOD of LC–ICP-MS may be not sufficient low, and because of this, ultrasonic nebulizer or pneumatic nebulizer with a desolvation system can be used for improving the LOD [14–16]. In the case of hydride-forming elements and Hg the LOD can be further improved by coupling VG to LC–ICP-MS. In this case, the volatile species generated from the analyte species present in the eluant can be introduced directly into the plasma.

Gas chromatography is advantageous with respect to sample transport efficiency to ICP-MS, which is nearly 100%, while less spectral interference is observed since the plasma remains dry [17]. Capillary GC has a high resolving power, making capillary-GC–ICP-MS an attractive technique for speciation analysis. GC–ICP-MS can be used for speciation of organometallic and organometalloid compounds, since they are sufficiently volatile or can be derivatized to produce more volatile species. Good peak resolution and low LOD are obtained using GC–ICP-MS.

Differently of LC, a fully heated interface is necessary for coupling GC to ICP-MS. The main requirement of any GC–ICP-MS interface is that the volatilized analytes remain in the gas phase during the transport from the GC column to the ICP. Therefore, any condensation of the analytes must be avoided to assure not only quantitative analyte transport but also the peak sharpness necessary for high resolution. This is usually achieved by heating the entire transferline to avoid any cold region. Two approaches are used to couple the transferline to the torch. In one of the approaches, the conventional spray chamber is removed and the transferline is inserted into the central channel of the torch. In this case, a carrier or make-up gas flow is added in order to obtain the typical carrier gas flow rate used in ICP-MS (about $1\,L\,min^{-1}$). Depending on the volatility of the analyte species, the degree of heating is varied. In the other approach, the end of the capillary column is coupled to the pneumatic nebulizer where the aqueous aerosol and the GC column effluent are mixed and then transported to the ICP. The exception of non-heated interface using is for compounds with low boiling point, which do not condense in any part of the transferline [18]. It is also recommended to add a small amount of oxygen to the make-up gas in order to prevent deposition of carbon (from the solvent present in the sample) on the interface of the ICP-MS instrument [17]. Additionally, variations in the plasma conditions can be observed as a result of the chromatographic effluent composition. However, this can be circumvented to some extent by constant monitoring the signal of Xe added to the make-up carrier gas flow [18].

The coupling of CE to ICP-MS is not as straightforward as LC and GC [19]. It requires a low dead-volume interface where the

solution flow rate is usually lover than 0.5 μL min^{-1}. The interface must provide a stable electrical connection to the exit of the capillary, preventing a laminar flow in the capillary, and offering high analyte transfer efficiency [8,19,20]. The nebulizers used to interface CE with ICP often cause suction or backpressure that produce a laminar flow in the capillary, compromising the electrophoretic separation. High efficiency interface is obtained by using a total consumption micronebulizer combined with a miniaturized low-dead-volume spray chamber [8,21–24]. Interfaces for CE–ICP-MS are commercially available, but homemade interfaces have been mostly used. The absolute LOD attainable by CE–ICP-MS is in the fg range. However, the LOD of the method is usually higher than this owing to the low sample volume used. Hence, depending of the sample, the concentration of metals and metalloids may not be detected by CE–ICP-MS. CE has a high resolving power and can separate ions of different charges but its major focus is macromolecules speciation as metalloproteins.

3. Applications of speciation

3.1. Arsenic species

Arsenic is a toxic metalloid element widely distributed in different environmental and biological systems. The toxicity of As depends on its chemical forms, whereas inorganic species are in general 100 times more toxic than organic species [25,26]. Studies dealing with As in humans have shown that exposure to the element may lead to cancer (in liver, kidney, bladder, prostate, lymphoid, skin, lung and colon) as well as other adverse health effects [27]. Humans are exposed to As mainly through ingestion of drinking water and food [28]. Organic As compounds are predominantly found in fruit, vegetables, seaweed, marine fish, and shellfish. Fish and shellfish can bioaccumulate AsB and AsC, organoarsenical compounds that are of low toxicity to humans. Synthetic organic As compounds are widely used and some of them present risk to human health. Methyl and phenyl derivatives such as MMA(III) and roxarsone are examples of synthetic As compounds [29].

Arsenic speciation in animal tissues has been studied in order to obtain more information about As metabolism. In humans, As(V) is reduced to As(III), which is generally metabolized to methylated As compounds and excreted *via* urine. MMA(V) and DMA(V) are the main products of the methylation process [28]. Arsenic speciation in urine has been considered a biomarker of recent exposure to the element [30], while As taken by the body can be found in hair that can be a biomarker for long term exposure [31]. Arsenic speciation in saliva is potentially useful for biomonitoring human exposure and studying arsenic metabolism [32]. Alike the speciation of other elements, a crucial requirement for obtaining reliable speciation information is to maintain the concentration of the original As species. Methods for stabilizing As species in liquid samples such as urine and blood or for extracting As species from solid biological tissues samples [30,33–35] need to be developed and validated.

Urine samples were usually kept in darkness at 4 °C [36] or 5 °C [37,38] or frozen until analysis. Sample preparation has usually involved homogenization at room temperature or thawing and homogenization at room temperature, followed by dilution, centrifugation and filtration [27,39], or only filtration [36,40,41], or only dilution [42]. Saliva was stored at −20 °C until analysis. The frozen saliva samples were thawed at room temperature, 3-fold diluted, sonicated and filtered [32]. Blood samples were separated into blood cells and cells were then washed with phosphate-buffered saline solution. The blood cells and plasma were stored at −80 °C until analysis. Higher molecular mass proteins in the plasma were separated by ultra-centrifugation and the filtrate analyzed [43]. Solid biological samples were washed or freeze-dried prior to As species extraction.

Procedures of As species extraction from biological materials included extraction with water at room temperature, centrifugation and filtration [44], or incubation in water at 90 °C followed by centrifugation [45–47], or enzymatic extraction at 37 °C in NH_4HCO_3 solution [48], or sonication in presence of water/methanol followed by centrifugation [49], or extraction with NaOH/methanol at 90 °C followed by centrifugation and filtration [50,51], or extraction using TMAH under heating at 60 °C prior to centrifugation [52], or extraction with cloroform/methanol/water and sonication [34], or sonication in water and centrifugation [53], or sonication in methanol/chloroform and centrifugation [54]. Additional extraction procedures are cited in the paper published by McSheehy and Mester [55].

LC–ICP-MS has commonly been used for As speciation. ICP-MS offers element specific identification since it converts the various arsenic compounds into As ion prior detection. Signal identification is achieved by matching the retention time of the standard and analyte and/or the sample spiked with standards. Several works report the application of LC–ICP-MS for As speciation in biological tissues and body fluids [27,31,39,43,49,50,54,56–58]. However, difficulties for identification and quantification of all As compounds found exist as a result of the great number of As species that are usually present in biological samples. No molecular information is obtained for a compound eluting with retention time not matching with that of the standard. Even though the resolution of LC methods are often sufficient for the numerous organoarsenicals present in biological samples, there is always a risk of misleading results due to the co-elution of unknown arsenic species with standards, which may lead to misidentification of signals. The identification of new arsenic species can only be achieved by using molecular specific techniques such as LC–ESI-MS, GC–ICP-MS, GC–TOF-MS and GC–EI-MS [37]. However, these techniques lack the quantitative analytical ability of LC–ICP-MS as the response of the detector is compound dependent [51].

Anion or cation-exchange column are frequently used for chromatographic separation of As species. Ionic strength, pH, temperature, mobile phase and presence of an organic modifier in the mobile phase affect species separation. Anion exchange stationary phases were used to separate As(III), As(V), MMA(V), DMA(V), and cation exchange to separate AsB, AsC, TMSO and Me_4As. This issue is extensively discussed in the literature [30,59–61].

Determination of As(III), As(V) and their methylated metabolites MMA(III) and DMA(V) in saliva collected from people exposed to As was possible by using LC–ESI-MS–MS and LC–ICP-MS [32]. Inorganic arsenic [As(III) and As(V)] and organic arsenic [MMA(V), DMA(V) and AsB] were determined in human urine using anion exchange in LC–ICP-MS. The presence of As species in urine of non-exposed and exposed individuals to the element, of a person after a failed suicide attempt with As_2O_3 ingestion and of individuals that received the antidote DMPS was investigated. For the non-exposed individual the toxicologically relevant As species in urine consisted of 81% DMA(V), 10% MMA(V) and 9% inorganic As. It was also observed that few hours after acute intoxication with inorganic As this distribution had changed dramatically and As(III) and As(V) were predominantly found in urine of people exposed to As. After treatment with DMPS, the total As concentration in the urine of individuals exposed to the element increased significantly, while MMA(V) and As(III) were the predominant species found [31]. A similar study was carried out to evaluate As exposure of people living in an area with soil naturally rich in As. LC–ICP-MS was used for As speciation analysis in the urine of the people and the main As species found were DMA, As(III) and MMA, while the DMA concentration was higher than those of MMA and As(III) [28]. Samanta et al. [39] used LC–ICP-MS and anion exchange for As spe-

ciation in urine of persons living in an area contaminated with As. AsB, DMA, MMA, As(III) and As(V) were the species detected.

The presence of arsenic metabolites in urine of sheep was investigated using LC–ICP-MS and LC–ESI-MS in parallel. DMA(V), DMAE and DMAA were found in the sheep urine [37].

Nail and hair were used to monitor As species concentration in humans living in an area potentially contaminated with As. LC–ICP-MS was used for determination of As species in aqueous extracts of fingernails and hair. The As species found in the fingernails were As(III), As(V), MMA(V), DMA(III) and DMA(V), while As(III), As(V), MMA(V) and DMA(V) were found in the hair. The authors concluded that DMA(III) in fingernails and DMA(V) in fingernails and hair could be used as biomarker to As exposure [45].

A LC–ICP-MS method was developed for As speciation in fish. Separation of eight species of As [As(III), As(V), MMA(III), DMA(V), AsB, AsC, TMAO and TeMA] were possible through a C_{30} reverse phase column. AsB, DMA(V) and TMAO were detected in the analyzed fish [53].

The LOD of As can be improved by coupling HG with LC–ICP-MS [41,46,47]. It enhances sensitivity up to 100-fold over the more commonly used pneumatic nebulization systems. Furthermore, HG reduces interferences caused by the mobile phase and sample. By using LC–HG–ICP-MS, the LODs of As(III), As(V), MMA(V) and DMA(V) are at ng L^{-1} level [30]. A comparative study about LC–HG–ICP-MS, LC–HG-AFS and LC–HG-AAS was conducted in order to determine inorganic As and its metabolites in urine. The species found were inorganic As, MMA and DMA. The authors observed that the results obtained using the three techniques were in good agreement [41].

Arsenic speciation can also be performed using HG–ICP-MS. A method based on HG–ICP-MS was developed for determining toxic As species (inorganic As, MMA and DMA) in urine from people exposed to As in drinking water. Under specific conditions of HG (1% $NaBH_4$, 0.2 mol L^{-1} HCl and 0.05 mol L^{-1} L-cysteine) the toxic As species were effectively separated from non-toxic As species. The concentrations of As species found using HG–ICP-MS were compared with those found using IC–ICP-MS and they were in agreement [36].

Arsenolipids are present in a wide range of biological systems in which they play a role in the biosynthesis of organoarsenic compounds. The presence of arsenolipids in canned cod liver was investigated. Arsenolipids were extracted and purified using a silica gel column. Ethyl acetate/methanol was used as eluant. Arsenolipids were identified and quantified using GC–ICP-MS, GC–MIP-AES, GC–EI-MS and TOF-MS. The results obtained by GC–ICP-MS and GC–MIP-AES showed the existence of several arsenic compounds in the extracts. The presence of arsenic-containing hydrocarbons ($C_{17}H_{37}AsO$, $C_{19}H_{41}AsO$ and $C_{23}H_{37}AsO$) in the sample was confirmed by GC–EI-MS and TOF-MS [51].

3.2. Mercury species

Mercury, a worldwide pollutant from natural or anthropogenic sources, can be globally transported and released to the environment [62]. Mercury is present in the environment as inorganic (mainly Hg^0 and Hg^{2+}) and organic (mainly MeHg) forms [63]. Due to their lipophilic characteristics and possibility of bioaccumulation, organic species of Hg are more toxic than the inorganic species of the element. Methylmercury is a well known toxic compound, a neurotoxin [64] that can be biomagnified across the trophic chain.

Mercury speciation in biological material has usually been performed by coupling LC [41,65–81] or GC [82–95] or CE [96–103] to ICP-MS. The LOD of Hg obtained using GC–ICP-MS is usually lower than that obtained using CE–ICP-MS or LC–ICP-MS. However, Hg speciation by GC–ICP-MS usually requires a derivatization step to convert low volatile species into more volatile species. Despite the derivatization step, Hg speciation analysis by GC–ICP-MS is in general simpler and faster in comparison with CE–ICP-MS or LC–ICP-MS. The employment of GC–ICP-MS for Hg speciation is also advantageous with respect to transport efficiency. In GC–ICP-MS all sample is vaporized and introduced into the plasma, differently of LC–ICP-MS in which the eluant from the separation column is introduced into the plasma *via* nebulization.

Solid-phase microextraction has been used for Hg speciation analysis by GC–ICP-MS, including specific applications for organomercury compounds [89–92,104]. Matrix separation, analyte preconcentration and measurement in absence of organic solvent are the main characteristics of SPME. Davis et al. [90] developed a method for the determination of MeHg in biological specimens using MAE and acetic acid for analyte extraction, followed by derivatization (ethylation) and headspace in combination with SPME–GC–ICP-MS. The LOD of MeHg was 4.2 pg g^{-1} (as Hg).

Isotope dilution and SSID lead to precise determination of Hg species [85,91,105]. ID and SSID also allow monitoring of species interconversion. Qvarnstrom et al. [93] determined MeHg, EtHg and Hg^{2+} in tissues of mouse treated with thimerosal. Stable enriched isotope standards of each Hg species were used for Hg determination using GC–ICP-MS. In a study conducted by Baxter et al. [82], MeHg was determined in animal and human blood. Isotopically enriched MeHg standard was used. Samples were digested with KOH or Hg species were simply extracted with KOH. The extraction procedure and ID were suitable for MeHg quantification in whole blood by GC–ICP-MS. The LOD of MeHg was 0.03 μg L^{-1} Hg.

When LC–ICP-MS is employed for Hg speciation, a RP column is commonly used. However, the organic solvent concentration in the mobile phase must be as low as possible in order to reduce carbon deposits on the ICP-MS instrument interface. In order to overcome the drawback caused by organic solvent, VG can be employed after the LC separation, leading only the volatile Hg species being introduced into the plasma [65,106]. The LOD of Hg is usually improved when VG is combined with LC–ICP-MS. However, depending on the experimental conditions, not all Hg species are transformed into volatile compounds.

Analysis of blood and hair is useful to verify human exposure to Hg. Determination of MeHg and inorganic Hg in blood and hair (extracted with mercaptoethanol, L-cysteine and HCl) by LC–ICP-MS is described by Rodrigues et al. [107] and Souza et al. [108]. LC–ICP-MS was also applied for Hg speciation in hair and certified fish, which were digested with TMAH [73]. Trümpler et al. [109] evaluated the effect of commonly used antidotes for detoxification of Hg. To do so, they analyzed whole blood using LC coupled to either ICP-MS and ESI-TOF-MS for detecting adducts formed among Hg species and thiols (cysteine and glutathione). Releasing of Hg in patients under treatment with the antidotes (2,3-dimercaptosuccinic acid and N-acetylcysteine) was observed.

A protection function of Se against toxic effects caused by Hg was found in some studies [76,110,111]. Li et al. [76] analyzed urine of volunteers from a mining area. The volunteers had been supplied with Se during 90 days. Speciation analysis of Hg and Se in the urine was simultaneously conducted using LC–ICP-MS. The authors observed that the concentration of Hg increased in the urine of the volunteers and suggested that Se could promote Hg excretion. The effect of Se and Hg in chicken liver was investigated by Cabañero et al. [111]. The animals were supplemented with Hg, MeHg and Se(IV) or Hg and MeHg for a period of 42 days. SEC–ICP-MS was employed for Hg speciation in the chicken liver cytosol. Different sub-cellular distribution and distribution pattern were observed for chicken supplemented with Se(IV), in comparison with those not supplemented with Se(IV), suggesting significant interaction between Hg and Se.

Interaction of Hg species with proteins is helpful for understanding Hg toxic effects. Speciation of Hg in salmon egg cells

was investigated by using surfactant-mediated LC–ICP-MS and subsequent separation of the protein fraction by SEC [57]. A C_{18} column coated with a bile acid derivative, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate), was used for species separation. Separation of large molecules (Hg bound to proteins) as well as small molecules or inorganic ions was possible.

High resolution, high efficiency, minor disturbance of the existing equilibrium between metal and metal–biomolecule complexes during separation, and short separation time for various analytes make CE suitable to study metal–biomolecule interactions. The potential applications of CE–ICP-MS for Hg speciation analysis and studies about biomolecules interaction with metals or metalloids were reviewed by Yin et al. [112] and Timerbaev [113].

3.3. Iodine species

Iodine is an essential element utilized by the thyroid gland in the biosynthesis of the hormones thyroxin – tetraiodothyronine (T4) and triiodothyronine (T3). Immune protection and antibody production depend on good thyroid function and the availability of T4 and T3 hormones [114]. While the total concentration of I in urine can reveal insufficient supply of the element, the concentration of I species are helpful for checking I metabolism or thyroid gland disfunction, which can be detected by changes of the normal concentrations of T3 and T4.

LC–ICP-MS has been used for I speciation in biological tissues or body fluids [115–120]. A short IC anion exchange column (50 mm length) was adequate for I^- and IO_3^- separation in human urine in only 170 s [121]. Simon et al. [120] used LC–ICP-MS for speciation of I in frog tissue. Quantification of I^-, T4, T3, reverse T3 (rT3), monoiodotyrosine and diiodotyrosine was possible in a single injection of sample. Other five compounds of I were also detected but could not be identified. In another work [115], the metabolic route of 2-, 3- and 4-iodobenzoic acids was investigated. These compounds were injected in mice and the metabolites produced were identified using LC–ICP-MS and LC–ICP-MS/ESI-MS. For LC–ICP-MS/ESI-MS analysis the eluant was split and conduced simultaneously to both ICP-MS and ESI-MS. Selected urine and bile samples were further analyzed using LC–ICP-MS in parallel with LC–MS for identification of the metabolites. Total I concentration was determined using ICP-MS and the excretion balance showed that all three iodobenzoic acids were rapidly excreted. Recoveries ranging from 95 to 105% of the administered doses were achieved within 24 h, indicating extensive metabolism. The major metabolites of all three iodobenzoic acids, glycine and ester glucuronide conjugates, were identified. Leiterer et al. [116] determined I^- and IO_3^- in bovine milk using LC–ICP-MS. An ion exchange column was used for I^- and IO_3^- separation and $Na_2CO_3/NaHCO_3$ buffer was used as mobile phase. The sum of I^- and IO_3^- contents found using LC–ICP-MS was different of the total content of I found using ICP-MS. The authors suggested the presence of organic I compounds in milk, correspondent to unidentified peaks in the chromatogram obtained.

Iodine is also used in labeling experiments for proteins quantification by ICP-MS and proteome studies. The principle of the method is to bind I to histidine and tyrosine in proteins [122]. Labeling with I is advantageous in comparison to S that is naturally present in proteins. Quantification of S is more difficult because of the high blank values, as a consequence of proteins separation using sodium dodecylsulfonate in CE.

3.4. Antimony species

Antimony compounds have different toxicity, bioavailability and reactivity. The element is potentially toxic even at low concentrations and has no biological function [123]. In general, elemental Sb is more toxic than its salts while inorganic species are more toxic than the organic [124]. Antimony (in contrast to As) is not detoxified via methylation in mammals.

Speciation of Sb in biological samples has been practically limited to Sb(III), Sb(V), mono-, di- and tri-methylated Sb and the complexes Sb(V)–lactate, Sb(V)–citrate and Sb(III)–glutatione [125]. Despite the toxicity of Sb, compounds of the element are widely used for Leishmaniasis treatment in tropical areas [126–131]. The classical treatment of human visceral Leishmaniasis involves the use of Sb(V). Efforts have been made to develop reliable methods to ensure the quality of the Sb-drugs used and also investigate the principle of their action in the human body [132]. Sb(III), which is very toxic, may be present as contaminant in the Sb-drugs and must be controlled.

Speciation of Sb is a challenging task because Sb compounds are unstable during the analytical process [123,133]. Quantitative extraction of Sb species from biological matrices is a long standing analytical challenge. Mild extraction procedures must be used in order to maintain the integrity of the Sb species. Solutions of methanol/water, citric acid, acetic acid, sodium hydroxide/EDTA/acetic acid, and chloroform–methanol/water/nitric acid are examples of extractants for Sb species. Simple stirring, or sonication, or microwave irradiation, or Soxhlet extraction can be employed to assist Sb extraction from biological materials. Another critical issue related to Sb speciation analysis is the unavailability of high purity Sb standards and certified reference materials [125,133].

The current methods of Sb speciation analysis in biological samples are focused on HG, LC, GC and CE (this in lesser extension) hyphenated with ICP-MS [133]. Hansen and Pergantis [125] reviewed the potential use of hyphenated techniques for Sb speciation in biological materials.

The employment of HG for Sb speciation in extracts of biological materials is well known. However, HG allows inorganic Sb(III) and Sb(V) speciation only. Basically, volatile stibine is generated from Sb(III) under mild acid conditions (pH > 2), whereas Sb(V) is only converted to stibine at higher acid concentrations (pH < 1). Sb(V) can also be determined by its pre-reduction to Sb(III) followed by stibine generation. In both cases, Sb(V) concentration is calculated by the difference of total Sb and Sb(III) concentrations. Antimony species present in biological samples can be derivatized to produce species with lower boiling point such as stibine, methylstibine, dimethylstibine and trimethylstibine that can be easily separated by GC. However, due to the low boiling point of these compounds they are usually cryotrapped prior introduction into the chromatograph [133]. Inorganic and methylated Sb compounds were identified in human urine using HG–LT-GC–ICP-MS [134]. Another procedure that can be used for Sb speciation is based on the collection of volatile Sb-hydrides using headspace and subsequent injection into a gas chromatograph. However, care must be taken when using derivatization by HG because severe conversion of the methylated Sb species may occur [135].

Antimony species separation by LC is usually based on the use of anion exchange resins, combined with a mobile phase containing complexing agents such as EDTA, phthalic acid, sodium citrate, or ammonium tartrate [133,136,137]. These complexing agents improve Sb(III) elution and also preserve Sb(III) during the chromatographic separation [138]. Some compounds of Sb may not be well separated or the retention by the anion exchange resin is irreversible. In order to achieve better Sb species separation, different anion exchange materials and mobile phase composition have been investigated [133]. Good separation of Sb(III) and Sb(V) is achieved using EDTA or phthalic acid with pH about 5.0 as mobile phase. On the other hand, mobile phase with higher pH and absence of complexing agent are required for trimethyl-antimony separation

[125]. In this way, separation of inorganic species and methylated Sb species are possible in only one run just by changing the mobile phase [139].

Separation of Sb compounds in urine was efficiently obtained using SEC and RP-LC (a C_{18} column was used). Complexes of Sb(V)–adenosine, Sb(V)–$(adenosine)_2$ and Sb(V)–citrate and non-complexed Sb(V) were separated in urine [140].

CE–ICP-MS and ESI-MS were also used for Sb speciation. However, the LOD of the CE–ICP-MS may not be sufficiently low to detect low concentrations of Sb species in biological samples [141]. ESI-MS is a complementary technique for Sb speciation analysis and useful for analyte identification since few Sb species standards are commercially available. This technique was used for identification of Sb species in urine [135]. The urine sample was properly diluted with water for analyte quantification using LC–ICP-MS. An anion exchange column (PRP-X100) was used, while the mobile phase was EDTA/phatalic acid, or ammonium formate/methanol, or ammonium formate/methanol/formic acid. The conclusion of the authors was that Sb(V) coordination complexes form extensively in certain biological matrices. They also concluded that Sb(V) complexes of high stability such as Sb(V)–citrate, Sb(V)–$(adenosine)_n$, and Sb(V)–$(lactate)_n$ ($n=1$ or $n=2$) are not detectable by most analytical methods based on HG volatilization and subsequent detection of stibines. For that reason, total Sb concentration may be underestimated in samples containing complexing ligands with high affinity toward Sb(V) [140]. An application of ESI-MS to study the kinetics of reduction of Sb(V) to Sb(III) involved in biological activity of Sb(V)-containing leishmaniases drugs is described by Ulrich et al. [138].

Ion chromatography and ICP-MS were used to investigate Sb accumulation and intracellular metabolism in the protozoan parasite *Leishmania donovani*, the major causative agent of visceral *Leishmaniasis*. Cell extracts were diluted with distilled water and aliquots from each preparation were analyzed using a PRP-X100 IC column. Sb(V) and Sb(III) were eluted from the column using 15 mM nitric acid and directly introduced into the plasma. The authors concluded that intracellular Sb metabolism occurred in both promastigote (the extracellular form) and amastigote (the intracellular form). The data obtained demonstrated that the interaction between Sb(III) and Sb(V) occurs intracellularly, within the parasite. The results also indicated that Sb(V) anti-leishmanial activity is dependent on its reduction to Sb(III) and it may or may not be enzymatic [142]. In a similar study some unidentified peaks were observed in the chromatograms of the cell samples, which could be organic Sb compounds [138].

Krachler and Emons [137] used LC–HG–ICP-MS and LC–USN–ICP-MS for Sb speciation in urine of occupationally exposed and non-exposed individuals. The only sample pretreatment involved dilution and filtration prior injection into the chromatograph. Depending on the concentration of the Sb species in the urine samples, they were diluted from 1+2 up to 1+49 with the mobile phase employed. A PRP-X100 column was used for Sb(V) and Sb(III) separation while EDTA was used as mobile phase. $TMSbCl_2$ and Sb(V) were separated using an anion exchange column and NH_4HCO_3/tartaric acid as mobile phase. Sb(V) was the predominant Sb species, followed by $TMSbCl_2$. Only ultratraces of Sb(III) were found. The sum of the concentrations of Sb(V), Sb(III) and $TMSbCl_2$ in urine samples ranged between 51 and 78% of the total Sb concentrations. Part of the Sb remained non-identified.

Lindemann et al. [143] used anion exchange and LC–ICP-MS to investigate the stability of Sb(III) and Sb(V) in fish and urine. Extraction of Sb compounds from spiked fish tissue was carried out using methanol/water and sonication, followed by centrifugation and decanting. The supernatant was evaporated to dryness and redissolved with water. It was concluded that methanol/water lead to partial conversion of Sb(III) and Sb(V) to new unidentified Sb species. In the case of urine, results indicated that under the investigated conditions the samples must be analyzed immediately after sampling if the content of Sb(V) is intended.

In the work reported by Foster et al. [144], LC–ICP-MS was used for Sb speciation in certified fish tissue. Different extractors were investigated. The study revealed that non-aggressive solvents such as water, diluted nitric acid, sodium hydroxide, and enzymes extracted highly variable amounts of Sb (2–84%). Antimony in biological tissues appeared to form very stable Sb species that are not readily extractable with solvents commonly used to extract metals and metalloids from animal tissues. Only traces of inorganic Sb were observed in the water extract.

3.5. Selenium species

Selenium has anti cancer properties and is one of the most investigated trace elements [145–147]. The biological pathways of Se have been extensively studied and the mechanisms of Se incorporation into proteins have been documented [148]. However, not all questions about the biological role of Se where answered up to now and biomethylation processes are still not sufficiently clear.

Accurate determination of organic and inorganic Se species in biological matrices is challenging because of unavailability of Se standards and lability of many organoselenium compounds [146,149]. Interesting and detailed reviews about Se speciation in biological matrices were published and this issue discussed [150–152].

LC–ICP-MS has frequently been used for Se speciation. Separation of Se species is possible using cation exchange [153–156], anion exchange [156–159], ion-pairing [160,161], RP [157,162–164], SEC [158,165,166] or AF [163,167,168]. Nevertheless, due to the wide variety of inorganic and organic Se compounds present in living organisms, it has not been possible to identify all compounds using LC–ICP-MS. As a consequence, speciation of Se using LC–ICP-MS is still limited to the availability of standards of Se species. For this reason, techniques such as ESI-MS and LC–ESI-MS-MS are needed to identify the unknown selenium species [169].

Despite interference of polyatomic species observed in Se determination by ICP-QMS, this type of instrument has been extensively used for Se speciation analysis. Interference caused by $^{40}Ar^{40}Ar^+$ and $^{40}Ar^{38}Ar^+$ over the most abundant Se isotopes (^{80}Se: 49.7% and ^{78}Se: 23.6%, respectively) increase the Se LOD. Elements such as Br and Cl present in biological matrices can also produce the interfering species $^{81}BrH^+$ and $^{40}Ar^{37}Cl^+$ that have the nominal mass of ^{82}Se and ^{77}Se, respectively. For selective removal of Br and Cl that would interfere on Se, selenoproteins in human serum were separated using AF or anion exchange prior to Se detection by ICP-MS [168,170]. SPE was used for pretreatment of biological fluids in order to improve the LOD of Se and overcome matrix interference [160,171]. Wrobel et al. [160] employed SPE for matrix removal and preconcentration of Se species in human urine. Currently, collision/reaction cell technology is used to circumvent the most common interferences caused by polyatomic ions on Se quantification by ICP-MS. Methane [159,172–174], CO [175], He [173], H_2 [161], O_2 [176], or combination of them are used as reactive or collision gases [77,177]. Although more expensive, ICP-SFMS [3] is also recommended since the disturbing isobaric of Se and polyatomic ions can be separated at the required mass resolution. LC–ICP-MS was applied for simultaneous determination of GPx, SelP and SeAlb in human serum [167,178]. In this case, ICP-SFMS was used in combination with a high-efficiency sample introduction system [179].

Juresa et al. [16] observed that the presence of volatile organoselenium compounds, especially DMSe and DMDSe, could increase ICP-MS responses relative to Se when conventional pneumatic nebulization was used for sample introduction into the plasma. This

effect was attributed to DMSe and DMDSe volatilization in the spray chamber, resulting in enhanced transport to the plasma. In this way, simple methods for Se species determination in biological fluids based on sample dilution can show overestimated results.

General aspects related to collection, processing and storage strategies for Se speciation analysis in clinical, nutritional and environmental applications are discussed by Wrobel et al. [180]. Enzymatic hydrolysis is one of the most frequent and efficient method of Se extraction from biological tissues [153,158,171]. Quijano et al. [171] described a procedure of Se species extraction from tuna fish and mussel tissues using enzymatic hydrolysis before analyte determination by LC–ICP-MS. A pre-extraction step with chloroform/methanol was carried out for fat removal. After enzymatic hydrolysis, the mixture was cleaned-up through a C_{18} cartridge to reduce matrix interference. The enzymatic hydrolysis was improved by sonication [181] or microwave irradiation [158]. Although enzymatic hydrolysis cannot distinguish SelP from GPx (both containing SeCys), when combined with SSID enzymatic hydrolysis it allows accurate quantification of SeAlb in serum that does not contain free SeMet [163]. According to Jitaru et al. [145], an advantage of enzymatic hydrolysis is the availability of SeMet standards, allowing accurate quantification of SeAlb in human serum; SeMet is quantitatively released from SeAlb as a result of the enzymatic hydrolysis. Two separation methods, SSID and enzymatic hydrolysis were used for SeMet, SeAlb, GPx and SelP Se speciation in human serum [163]. A RP column was used to separate SeMet whereas SeAlb, GPx and SelP in untreated serum sample were separated using AF.

The stability of Se species in biological matrices such as oyster tissue [153], human urine [157,162] and serum [165] was evaluated using LC–ICP-MS. Total Se as well as SeMet and TMSe remained stable for at least 12 months in oyster tissue under different temperatures (−18, +4 and +20 °C). On the other hand, Se species in enzymatic extracts stored at 4 °C were stable for 10 days only [153]. The studies suggested that urine samples should be cooled immediately after collection and kept at 4 °C for up to 2 weeks [157], or up to 4 weeks if kept at −20 °C [162], with minimum Se species loss. Palacios and Lobisnki [165] used SEC–ICP-MS to evaluate the stability of Se compounds in human serum. According to the authors, loss of Se in the protein fraction was observed after 15 days of storage at 4 °C. Most significant losses were observed for SeAlb and GPx, in addition to SeCys degradation.

Speciation of Se using GC–ICP-MS [182–186], CE–ICP-MS [187,188] and GE [189] is also reported. Scanning of electrophoretic gels by LA–ICP-MS is reported in Refs. [189,175]. A combination of headspace, SPME and GC–ICP-MS was used for DMSe and DMDSe determination in urine [184] and human lymphoma cells [185]. The presence of volatile Se species at their natural occurrence level in urine was reported for the first time by Bueno and Pannier [184]. Researchers have found selenite [161,190] and selenate [190] in human urine. The presence of selenite in urine was attributed to degradation of organoselenium compounds during storage [165]. Kuehnelt et al. [191,192] observed the presence of TMSe at trace levels as a common Se metabolite in human urine.

Ohta et al. [183] studied the behavior of ^{76}Se-SeMet, ^{77}Se-MeSeCys, and ^{82}Se-MeSeA, orally administered to mice. GC–ICP-MS and LC–ICP-MS were used for the determination of Se species in the urine of the mice. The authors observed that the Se tracers were metabolized and excreted *via* urine. The order of concentration in urine was ^{76}Se-MeSeCys > ^{82}Se-MeSeA > ^{77}Se-SeMet. Discrepancies between total Se determined by ICP-MS and the sum of Se species determined by LC–ICP-MS were observed because unknown Se species could not be quantified.

New Se compounds were recently discovered. One of them, 2-selenyl-Nα,Nα,Nα-trimethyl-L-histidine, named "selenoneine", was described by Yamashita and Yamashita [193] as a dominant Se specie in tissue and blood of bluefin tuna. Selenium speciation was carried out by LC–ICP-MS, whereas "selenoneine" was characterized by nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). Gabel-Jensen et al. [176] reported the presence of a Se-S-aminoacid, S-(methylseleno) cysteine, in mammalian cells model spiked with MeSe-aminoacid.

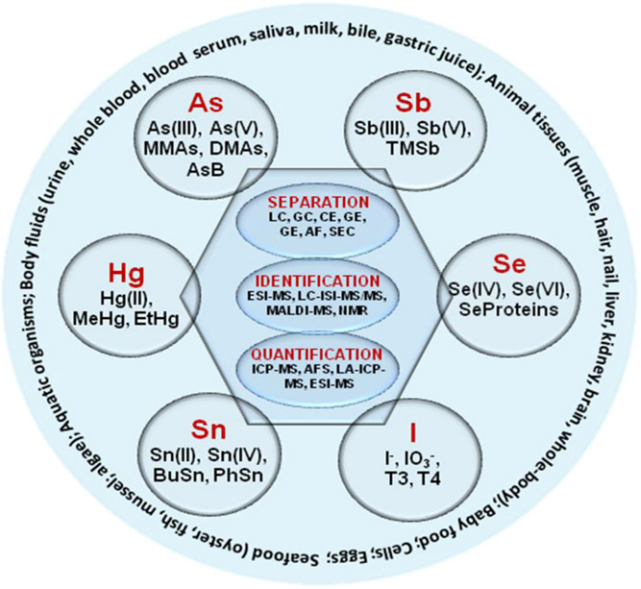

Fig. 1. Element speciation in biological matrices and techniques employed.

It has been demonstrated that most biochemical functions of Se in humans are mediated by SeProt, in the form of SeCys. The assessment of Se status in humans is therefore important. Consequently, the development of reliable analytical methods for the determination of Se species, particularly SeProt, is currently an imperative demand. In this way, the most critical topic related to SeProt determination in biological fluids is the method validation. Accurate quantification of Se bio-species is difficult because only few standards are available. However, external calibration with surrogate standards or species unspecific (on-line or post-column) ID can be used for SeProt quantification by ICP-MS [145,194,195]. Chromatographic resolution and detection of Se species were improved by using on-line ID and nano-LC–ICP-MS [10]. Development and advances of interfaces for μLC–ICP-MS and nano-LC–ICP-MS were reported by Schaumlöffel [6,8,11,13].

3.6. Tin species

Inorganic compounds of Sn [Sn(II) and Sn(IV)] are recognized as non-toxic, contrarily of organic compounds of the element that are toxic. Most of the OTs are from anthropogenic origin, with the exception of methyltin, which can also be produced by biomethylation. OTs are biocides and used as miticides, molluscicides, nematocides, ovicides, rodent repellants, wood preservatives and antifouling paints. TBT, TPhT and TCHT are the most used compounds. TMT is widely used in polyvinyl chloride industry, leading to acute intoxication related to neurological disorders. The toxicity of OTs to life depends on the type of alkyl groups and degree of substitution. TBT and TPhT even at low concentrations are very toxic to aquatic life. The less substituted butyltin (DBT and MBT) and phenyltin (DPhT and MPhT) compounds are less toxic, but are still of environmental concern [196].

Depending on the species involved, LC–ICP-MS [197] or GC–ICP-MS [95,198] can be used for OTs speciation. GC–ICP-MS combines high separation potential and sensitivity/specificity,

Table 1
Speciation of As, Hg, I, Sb, Se and Sn in animal tissue and body fluids.

Species	Sample	Separation/detection technique	Remarks	Ref.
Arsenic				
As(III)/As(V)/DMA(V)/ MMA(V)/DMA(III)/MMA(III)	Human saliva	LC–ICP-MS/LC–ESI-MS–MS	LODs: 0.03 μg L^{-1} for As(III), MMA(III), and DMA(V); 0.05 μ. g L^{-1} for MMA(V); 0.08 μg L^{-1} for As(V); and 0.1 μg L^{-1} for DMA(III); As(III), As(V), MMA(V) and DMA(V) were detected	[32]
As(III)/As(V)/DMA/MMA/ AsB/TMAO/AsC/TMAs	Human urine	LC–ICP-MS	LODs: 3–6 μg L^{-1} for cationic As and 7–10 μg L^{-1} for anionic As species; As(III), MMA, DMA, As(V), AsB, TMAO, AsC, TMAs and two unknown As species were detected	[34]
AsB/As(III)/As(V)/MMA/DMA	Human urine	HG–ICP-MS IC–ICP-MS	LOD: 6 ng L^{-1}; AsB, As(III), As(V), MMA(V), MMA(III), DMA(V) and DMA(III) were detected	[36]
DMA(V)/DMAE/DMAA	Sheep urine	LC–ICP-MS ESI-ICP-MS	DMAA and DMAE were detected; identification of arsenosugar metabolites	[37]
As(III)/As(V)/DMA(V)/MMA(V)/AsB	Human urine	LC–ICP-MS	LOQ: < 0.1 μg L^{-1}; As(III), As(V), MMA(V), DMA(V) and AsB were detected	[38]
As(III)/As(V)/DMA/MMA/AsB	Human urine	LC–ICP-MS	LODs: 0.01–0.04 μg L^{-1}; As(III), As(V), MMA, DMA and AsB were detected	[39]
As(III)/As(V)/DMA/MMA/AsB	Human urine	RP-LC–ICP-MS	DMAs(V), MMAs(V) and MMAs(III) were the major metabolites found	[40]
As(III)/As(V)/DMA/MMA/AsB	Human urine	LC–ICP-MS	LODs: 0.3 μg L^{-1} (AsB and MMA), 0.4 μg L^{-1} (As(III), As (IV) and DMA); As(III), As(V), DMA, MMA and AsB were detected	[42]
As(III)/As(V)/DMA/MMA/AsB	Human blood cells and plasma	LC–ICP-MS	Patients with promyelocytic leukemia (APL) treated with As_2O_3 were investigated; As(V), As(III), MMA, DMA and AsB were detected in blood and plasma; total As in blood cells was 4–10 times higher than in plasma	[43]
As(III)/As(V)/DMA/MMA	Human nail and hair	LC–ICP-MS	As(III), As(V), MMA(V), DMA(III) and DMA(V) were detected in nail, whereas As(III), As(V), MMA(V) and DMA(V) were detected in hair	[45]
As(III)/As(V)/DMA/MMA	Human hair	LC–HG–ICP-MS	As(III), DMA(V), MMA(V) and As(V) were detected	[46]
As(III)/As(V)/MMA/DMA/ AsB/TMAsO/AsC/TMAs	Human urine and fish tissue	LC–ICP-MS LC–HG–ICP-MS	LODs: 0.067–0.34 μg L^{-1} of As using LC-ICP-MS and 0.016–0.075 μg L^{-1} using LC–HG–ICP-MS; As(III), As(V), MMA, DMA and AsB were detected in urine, whereas As(III), As(V), MMA, DMA, AsB and TMAO were detected in fish	[47]
AsB/DMAA/MMA	Baby food	LC–ICP-MS	LODs: 0.9, 1.3, 2.6 and 5 μg L^{-1} for AsB, DMA, MMA and inorganic As, respectively; AsB was the main species detected	[48]
Arsenous acid–glycerol–ribose/DMA/MAA–Phosphate–ribose/arsenic acid/sulfonate–ribose/sulfate–ribose	Sea weed	LC-ICP-MS	LOD: 0.005 mg kg^{-1} for As compounds; As-sulfate-ribose, As-sulfonate-ribose and As- phosphate-ribose were the main species detected	[49]
Thio-arsenosugar	Marine shellfish	IC–ICP-MS IC–ESI-MS/MS	2,3-Dihydroxypropyl-5-deoxy-5-dimethylarsinoyl-b-D-riboside were the main species detected	[52]
As(III)/As(V)/MMA/DMAA/ AsB/AsC/TMAO/TeMA	Fish tissue	LC–ICP-MS	LOD: 0.2 ng mL^{-1} for each As species; AsB, DMA and TMAO were detected	[53]
As(III)/As(V)/MMA/DMA/AsB	Fish tissue and human urine	LC–ICP-MS	LOQ: 44, 56, 94, 64, 66 ng L^{-1} for As(III), As(V), MMA, DMA and AsB, respectively; acidification of sample prior to its injection was used to modify the retention of early eluting species (As(III))	[54]
As(III)/As(V)/MA/DMA/ AsB/AsC/TMAO/TeMA	Oyster tissue	LC–ICP-MS	Evaluation of MAE for arsenic species; arsenosugar was fragile and adequately transmuted to DMA (100%), AB and AC to TMAO (100%) when 450 W microwave power was applied for 15 min; arsenosugar was the principal As species in oyster tissue followed by AB and DMA; inorganic As, MA, AC, TMAO, TMI and unknown compounds were the minor constituents	[56]
As(V)/MMA/DMA/AsB	Body fluids (plasma, bile and urine)	LC–ICP-MS	LOD: 0.14–0.33 μg L^{-1} As for all species; AsB, DMA(V), MMA(V), and As(III) were detected in plasma; As(III), MMA(III), conjugated As(III)-GS, MMA(III)-GS and glutathione (GSH) in bile while the main specie found in urine was DMA(V)	[57]

Table 1 (*Continued*)

Species	Sample	Separation/detection technique	Remarks	Ref.
As(III)/As(V)/MMA/DMA	Sea weed	LC–HG–ICP-MS	It was observed that the four major arsenosugars found were hydride-active	[61]
Mercury				
Hg^{2+}/MeHg	Fish tissue	FI–HG–ICP-MS	LODs: 2.52 pg for Hg^{2+} and 3.24 pg for MeHg; species separation in a micro column filled with polyaniline and pH-selective elution were used	[65]
Hg^{2+}/MeHg	Human hair	LC–ICP-MS	LODs: 4 ng L^{-1} for Hg^{2+} and 10 ng L^{-1} for MeHg; cloud point extraction was used	[66]
Hg^{2+}/MeHg/PhHg	Human hair and fish	LC–ICP-MS	LODs: 6 ng L^{-1} for Hg^{2+}, 13 ng L^{-1} for MeHg and 8 ng L^{-1} for PhHg; pre-concentration using cloud point extraction	[67]
Hg^{2+}/MeHg/total Hg	Aquatic invertebrate organism	ID–GC–ICP-MS	Low sample amount (<10 mg); LODs: 1.44 pg for MeHg and 10.8 for Hg^{2+}	[69]
MeHg/Hg^{2+}	Fish tissue	LC–ICP-MS	LODs: 0.8 μg L^{-1} for Hg^{2+} and 0.7 μg L^{-1} for MeHg; Bi^{3+} was used as internal standard	[70]
MeHg/Hg^{2+}	Fish tissue, mussel tissue, bovine liver, prawn	LC–ICP-MS	LOD: 0.2 μg L^{-1} for Hg^{2+} and MeHg	[72]
MeHg	Fish tissue and human air	LC–ICP-MS	LOQ: 0.5 μg g^{-1} Hg	[73]
MeHg/Hg^{2+}	Fish (muscle and liver)	LC-ICP-MS	LOD: 1 pg for Hg^{2+} and 1.6 pg for MeHg	[74]
Hg-proteins	Rat brain (cytosol)	ID–SEC–ICP-MS	Enriched MeHg was administrated to pregnant rats; the distribution patterns of brain cytosol in dam and pup rats were similar, whereas Hg content in the two observed proteins were different	[75]
Hg-proteins	Salmon egg cell cytoplasm	LC–ICP-MS	Hg in salmon egg cell cytoplasm presumably binds with proteins containing SeCys and/or cysteine residues in proteins; a column coated with a bile acid derivative was used	[79]
MeHg	Whole blood	ID–GC–ICP-MS	LOQ: 0.03 μg L^{-1}; alkaline extraction was used	[82]
MeHg	Fish and seafood tissues	ID–GC–ICP-MS	LOD: 1.4 ng g^{-1}	[85]
Hg^{2+}/MeHg	Fish tissue	GC–ICP-MS	Total Hg in shark fillets ranged from 0.9 to 3.6 μg g^{-1}, more than 94% of Hg in the form of MeHg; DBT, dipentyltin and xenon were used as internal standards	[88]
Hg^{2+}/MeHg	Fish, oyster and mussel tissues	SPME-multicapillary-GC–ICP-TOFMS	LODs: 1.31 pg g^{-1} for Hg^{2+} and 1.97 pg g^{-1} for MeHg a carboxen/polydimethylsiloxane fiber was used	[89]
MeHg	Fish and oyster tissues, seabird eggs	SPME–GC–ICP-MS	LOD: 4.2 pg g^{-1}; a polydimethyl siloxane-coated silica fiber was used	[90]
MeHg	Fish tissue	SPME–GC–ICP-MS	LOD: 2.1 ng g^{-1}; SSID calibration was used	[91]
Hg^{2+}/MeHg/EtHg	Fish tissue	CE–ICP-MS	LODs: 0.2 pg for Hg^{2+} and 7 pg for MeHg; on-line continuous volatile species generation with $NaBH_4$ increased sensitivity	[101]
Hg^{2+}/MeHg	Fish tissue	ID–MC–ICP-MS	Evaluation of equilibration in ID; complete equilibration of MeHg between the liquid and solid phases was achieved with a mixture of H_2O/CH_3OH/2-mercaptoethanol; quantitative results were obtained, even though only 53% of the analyte was extracted	[105]
Hg^{2+}/MeHg	Human blood	LC–ICP-MS	LODs: 0.25 μg L^{-1} for Hg^{2+} and 1 μg L^{-1} for MeHg; MeHg in blood from people exposed to MeHg from fish consumption ranged from 20.3 to 85.1 μg L^{-1}	107
Hg^{2+}/MeHg/EtHg	Human hair	LC–ICP-MS	LODs: 15 ng g^{-1} for Hg^{2+}, 10 ng g^{-1} for MeHg and 38 ng g^{-1} for EtHg; MeHg in hair from people exposed to MeHg from fish consumption ranged from 2.23 to 3.12 μg g^{-1}	[108]
Hg^{2+}/MeHg/Hg-Thiol	Human blood and plasma	LC–ICP-MS and LC–ESI-TOF-MS	Adducts between Hg species and physiological thiol groups were observed	[109]
Hg^{2+}/MeHg	Chicken liver cytosol	SEC–ICP-MS	Interaction between Hg, Zn and Se occured when MeHg and Se were co-administrated; chicken were exposed to Hg^{2+} and MeHg added to feed with or without Se supplementation	[111]

Table 1 (*Continued*)

Species	Sample	Separation/detection technique	Remarks	Ref.
Iodine				
2-, 3- and 4-iodobenzoic acids	Rat bile and urine	LC–ICP-MS and LC–ESI-MS	2,3 and -4-iodobenzoic acids were administrated (*via* intraperiatonial); iodobenzoic acids metabolites (glycine and ester glucuronide conjugates) were identified in urine; amounts of unchanged iodobenzoic acids excreted were low	[115]
I^-/IO_3^-	Bovine milk	IC–ICP-MS	LOD: 1 μg L^{-1} (as I); iodine was present mainly as I^- (89%); the IO_3^- concentration was low; organic-bound iodine was presumably present; alkaline extraction was used	[116]
I^-/mono-iodothyrosine/di-iodothyrosine/tri-iodothyronine/reversed tri-iodothyronine (T3)/and thyroxin (T4)	Human urine	LC-ICP-MS	LODs: 0.08–1.5 μg L^{-1}; I^- was de predominant species found; T3 and T4 were present only in trace concentrations; two unknown I species were observed; urine was diluted with tetramethylammoniumhydroxyde for total I determination	[117]
T4/T3/reverse tri-iodothyronine (rT3)/monoiodotyrosine (MIT)/diiodotyrosine (DIT)	Zebrafish and clawed frog (whole-body homogenate)	LC–ICP-MS	Hormones and total I concentration were higher in zebrafish male, whereas hormone precursors where higher in male, indicating high hormone synthetic activity for male; T3 and T4 concentrations in zebrafish were higher than those found in tadpole of clawed frog	[120]
Antimony				
Sb(V)/Sb(III)	Human/blood, plasma, urine and hair	IC–ICP-MS	LOD: 0.16 μg L^{-1} for both Sb(V) and Sb(III); N-methyl meglumine antimoniate was administrated to patients with leishmaniasis; the drug conversion occured in vivo producing Sb^{5+} and Sb^{3+}; EDTA was used as mobile phase	[132]
Sb(V)/Sb(III)/$TMSbCl_2$	Human urine	LC–ICP-MS LC–HG–ICP-MS	LODs: 20 ng L^{-1}, 12 ng L^{-1} and 8 ng L^{-1} for Sb(V), $TMSbCl_2$ and Sb(III), respectively; Sb(V) was the predominant Sb species found in urine of occupationally exposed subjects, followed by $TMSbCl_2$; only ultratraces of Sb(III) were detected	[137]
Sb(V)/Sb(III)	Amastigode/promastigode cells of *Leishmania donovani*	IC–ICP-MS	LODs: 0.06 μg L^{-1} for Sb(V) and 0.29 μg L^{-1} for Sb(III); unidentified Sb species were observed (presumably, organic Sb compounds)	[138]
Sb(V)–citrate	Human urine	LC–ICP-MS LC–ESI-MS/MS	Evaluation of Sb(V)–citrate complexation; Sb(V) coordination complexes can be extensively produced in certain biological matrices	[140]
Sb(V)/Sb(III)	Amastigode/promastigode cells of *Leishmania donovani*	HG–ICP-MS IC–ICP-MS	Interaction of Sb(III) and Sb(V) occured intracellularly in *Leishmania donovani*; Sb(V) anti-leishmanial activity is dependent on its reduction to Sb(III); intracellular Sb metabolism occurred in both promastigotes (the extracellular form) and amastigotes (the intracellular form) of *Leishmania donovani*	[142]
Selenium				
Selenomethionine/Selenocysteine	Human serum	ID–LC–ICP-MS	LOD: < 0.5 ng g^{-1}; SeCys and SeMet were determined; SSID and enzymatic digestion were used; SEC was used for serum samples fractionation	[194]
Selenomethionine/Selenocysteine	Lamb blood	Ion pairing-LC–ICP-MS	LOD: about 0.02 μg g^{-1}; selenoaminoacids were proteolytic released from blood proteins and separated by SEC; SeCys and SeMet were detected	[195]
Seleninopeptides	Selenomethionyl calmodulin	nano-LC–ICP-MS	LOD: 40 fg; development of an interface for nano-LC-ICP-MS, allowing introduction of mobile phases containing up to 90% of acetonitrile into the ICP; selenopeptides in tryptic digest of selenomethionyl calmodulin were determined	[10]
Tin				
MBT/DBT/TBT	Fish tissue and seafood	ID–GC–ICP-MS	LODs: 0.3 ng g^{-1} for MBT and TBT, and 1.2 ng g^{-1} for DBT; a broad variation in the butylated Sn fraction (in the range of 0.3–49%) was observed	[85]

Table 1 (*Continued*)

Species	Sample	Separation/detection technique	Remarks	Ref.
TMT/DMT/MMT/MBT/DBT/TBT	Fish, oyster and mussel tissues	SPME-multicapillary-GC–ICP-TO-FMS	LODs: 0.30–0.99 pg g^{-1}; seven SPME fibers were compared, whereas a 65 μm polydimethylsiloxane/divenylbenzene fibber offered the best overall extraction efficiency	[89]
MBT/DBT/TBT	Simulated gastric/intestinal human fluids	ID–GC–ICP-MS	Simulation of digestion of Sn species in mussel tissue medium; the most significant degradation was observed for DBT, producing MBT; TBT was solubilized but not degraded; SSID was used	[199]
MPhT/DPhT/TPhT	Mussel and fish tissues	SSID–GC–ICP-MS	Extraction procedures for the speciation analysis of PhT compounds were evaluated; acidity of extractant, presence of complexing reagents, and use of ultrasonic agitation or microwave were found to affect the degradation of PhT compounds	[200]
MMT/DMT/TMT	Human urine	LC–ICP-MS	TMT was detected in urine of individuals exposed to dimethyltin dichloride	[201]
MMT/DMT/TMT	Mouse urine	LC–ICP-MS	Conversion of DMT to TMT was detected	[202]

making GC–ICP-MS attractive for OTs speciation in biological tissues. Nevertheless, due to low volatility of OTs (except the tetra substituted species that are volatile) a derivatization step (usually ethylation) is necessary in order to produce volatile compounds [199]. The major challenge of OTs speciation using GC–ICP-MS is the transport of the gaseous compounds from the gas chromatograph to ICP. Condensation of OTs can easily occur and a fully heated interface positioned as close as possible to the ICP is necessary. The cooling of some parts of the transferline can produce tailed-chromatographic peaks or complete condensation of OTs, especially TPhT species that have higher boiling point. Different interfaces were proposed for coupling GC to ICP-MS and some of them are already commercially available [200].

Tin has ten naturally occurring isotopes which facilitates the analysis using ID. Rodríguez-González et al. [201] investigated the solubilization of butyl tin species in a simulated human digestion medium and further speciation analysis of MBT, DBT and TBT using ID–GC–ICP-MS. SSID was used for monitoring the degradation of butyltin species. A solution containing each butyltin species was added to a simulated gastric or intestinal fluid for digestion in presence or absence of mussel tissue matrix. The most significant degradation was observed for DBT, producing MBT. The authors observed that TBT, the most toxic investigated species, was solubilized but not degraded.

OTs, mainly PhTs, are unstable and usually degraded by ultraviolet radiation, biological and/or chemical cleavage. The degradation of PhTs in biological tissues was evaluated by Van et al. [202] using SSID and GC–ICP-MS. Five different extraction procedures and two ethylation routes were compared using multi-isotope spikes of ^{118}Sn-enriched MPhT, ^{122}Sn-enriched DPhT and ^{124}Sn-enriched TPhT. The kind of extractant (acid, or base, or organic solvent), presence of complexing agents and the application of microwave radiation or sonication affected the degradation of PhTs. The use of SSID was useful to elucidate the degradation routes of PhTs in biological tissues, opening new possibilities for accurate speciation analysis of PhTs.

Speciation analysis of alkylated species of Sn can be performed by LC–ICP-MS. However, only few works describe the application of LC–ICP-MS for biological tissues [203–205]. The major advantage of LC is that derivatization is not needed, simplifying the speciation analysis. Besides, hyphenation of LC with ICP-MS is already well established. On the other hand, the introduction of the mobile phase (rich in salts and/or organic solvent) into the plasma is problematic and needs to be controlled. LC–ICP-MS was used to investigate the presence of alkylated Sn species in human urine from a patient exposed to DMT [201]. Ammonium formate/formic acid was used as mobile phase. After analysis by LC–ICP-MS an unknown peak was observed in the chromatogram. ESI-MS was used to identify this peak and the results showed that it corresponded to TMT. This species was found in the urine as a consequence of biotransformation of DMT in the body.

The present review is summarized in Table 1 and Fig. 1.

4. Conclusions

The following remarks can be drawn with respect to As, Hg, I, Sb, Se, and Sn speciation in biological materials and body fluids.

i. Hyphenated techniques such as LC–ICP-MS and GC–ICP-MS have been more employed for separation and detection of As, Hg, I, Sb, Se, and Sn species. However, due to molecular diversity of the elements, mainly As and Se, employment of other techniques for the identification of species is necessary.
ii. The knowlegde in speciation analysis has expanded dramatically in the last decade, mainly due to the development of new analytical techniques. In this context, ICP-MS combined with different separation techniques has played an important role.
iii. Despite the advances in instrumentation, quantification and identification of several species of As, Hg, I, Sb, Se, and Sn are still challenging due to the lack of standards for speciation analysis. Never less, advances in ID offer the possibility of more accurate results.
iv. In general, increased attention has been given to quality control with respect to precision and accuracy in speciation analysis. However, there are still few certified reference materials for speciation owing to difficulties associated mainly with the stability of the species.
v. Sample preparation procedures have also improved in recent years. Procedures (extraction/solubilization combined with stirring, microwave irradiation and sonication) were proposed for preparation of biological samples, in order to preserve the species of interest and also achieve quantitative extraction of them. However, quantitative extraction of some species is still not possible.
vi. Speciation analysis has evolved, through the determination of simple molecules and oxidation states of elements to identification and quantification of more complex species. To this end, besides the knowledge acquired in this area, improvement and

development of new techniques based on MS have been decisive.

vii. Studies on speciation analysis tend to identification and quantification of new biomolecules containing different heteroelements. In this context, ICP-MS combined with different separation techniques will be important mainly with respect to the quantification of heteroelements in molecules.

viii. Molecular mass spectrometry, including LC–ESI-MS, FTICR-MS and MALDI-TOF-MS will be increasingly important, especially for identification of macromolecules such as metalloproteins. Application of NMR in speciation analysis will also increase in view of the importance of this technique in obtaining structural information of molecules.

ix. The improvement of miniaturized separation techniques such as μLC, nano-LC and CE are necessary for chemical speciation analysis in biological materials, especially for separation of macromolecules containing heteroelements present in low concentrations in small amount of sample. Difficulties in coupling μLC, nano-LC and CE to ICP-MS are still found. The main difficulties are the pumping of solutions with flow rate at nL min^{-1}, injection of small sample volume, high pressure in the separation system, back pressure, plugging of capillaries, which affect the stability of the analyte signal.

In summary, speciation analysis (including As, Hg, I, Sb, Se, and Sn in biological tissues and body fluids) is still an increasing area of research, integrated with different fields of knowledge such as chemistry, biology, clinical chemistry, medicine, toxicology and occupational health. Several benefits from speciation data can be used keeping in mind that biological activity, toxicity and metabolic pathways of the elements depends on element species.

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