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FEATURE ARTICLE

Preconcentration, speciation, and determination of key elements in biological samples in Latin America

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Introduction

For many years, the biological samples used in diagnosing trace element deficiency or excess have been blood, serum, plasma, and urine. More recently, saliva, hair, and nails have attracted the attention of researchers owing to their noninvasive collection.

The concentrations of trace elements in biological samples are far below those achieved directly by most atomic or plasma-based techniques and the detection power needs to be improved by the use of a preconcentration step. In this connection, solid phase extraction (SPE) makes possible the concentration and purification of analytes from solution by their sorption onto a solid sorbent, also improving detection limits. The choice of the solid sorbent and its suitability for speciation analysis are of prime importance. For its simplicity and low cost, this method was, and still is, very much used by different research groups in Latin America as is illustrated in the literature.

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In the scientific community, the field of elemental speciation has been widened in the last few years owing to developments in instrumentation that can determine the molecular form of elements in real samples. More recently, research groups in Latin America have conducted speciation analysis using different approaches. Nonchromatographic methods such as SPE employing selective adsorption materials have been extensively used in speciation applied mostly to elemental speciation according to the oxidation state.

The challenge to determine metallic species in biological samples resides mainly in the complexity of these types of matrices as well as the low concentrations of target elements. Analytical chemistry has provided many solutions to this end, thanks to the development of innovative, sensitive, and selective methods. However, in recent years the necessity to identify, separate, and quantify various forms, not only inorganics, in which a chemical element may occur in matrices as diverse as biological materials arose, and this issue has put analytical chemistry in the spotlight again. Advances in speciation analysis have led to a new discipline, namely, metallomics, which is related to genomics, proteomics, and metabolomics interfaced by analytical chemistry.

Considering the above discussion, this review provides a composite picture of the progress, limitations, and research conducted in Latin America laboratories over the last 20 years and covers developments in elemental determination in biological samples with clinical interest, through speciation to emerging studies in metallomics [1].

A composite picture

To estimate in a quantitative and impartial fashion the well-established trend and tendencies in Latin American countries, we conducted a survey of the literature in this field. Rather arbitrarily, our survey took into consideration only information obtained from the Scopus database. The

keywords applied in our search were "biological samples," "determination," "speciation," and "preconcentration," and we applied filters for Latin American countries. The survey was based on the last 20 years of progress in the determination and preconcentration of elements of clinical interest in biological samples as well as the separative techniques used for speciation analysis. According to our search, in terms of the geographical location of the countries where the studies were conducted, the ranking in order of the most studies was as follows: Brazil > Argentina > Mexico > Venezuela > Chile.

The growth in the number of publications that has contributed to the determination of trace elements and their species in biological samples in Latin America makes it impracticable to cite, even briefly, all the contributions that have enriched the relevant literature to date. Hence, only a few selected representative contributions published mainly in this field are reported.

Biological matrices

In recent years, the analysis of key elements in human tissues has gained great interest owing to the role that these elements play in biochemical and physiological processes. Although trace elements constitute a minor part of living tissues, they are important for vital processes. Some metals, usually present in proteins, enzymes, and cellular membranes, are essential for normal physiological function.

Typical biological samples for trace element determination are hair, urine, serum and blood, colostrum and human milk, and saliva. Each of them exhibits different characteristics and needs a specific sample treatment. Table 1 depicts different samples analyzed, elements determined, and the analytical techniques employed by Latin American researchers. A brief description of the biological matrices studied is as follows:

- Hair: The determination of metal contents in human hair can be used either as an index of exposure to potentially toxic elements (poisoning) or as information on the health condition of an individual.
- Urine: For specific workplaces, human urine is a useful indicator of exposure to metals.
- Blood and serum: These are the ideal matrices owing to their contact with the whole organism.
- Colostrum and human milk: The study of the composition of human milk has attracted interest, since it represents the most suitable pattern of nutrients for infants.
- Saliva: Its usefulness relies on the direct contact of saliva, and also gingival crevice fluid, with the blood serum, offering a rapid and cheap method, which is also less traumatic for the patient.

The analysis of biological tissues often requires decomposition of the solid sample and its transformation into a homogeneous liquid phase before analysis. The next section will describe different sample treatments, strategies, and determination techniques used by Latin America researchers for analysis of biological samples.

Sample treatment of biological samples prior to trace element determination by atomic spectrometric techniques

Wet digestion procedures in open or closed vessels with microwaves have been very much used in the treatment of a variety of samples. Nevertheless, microwave-assisted digestion has several drawbacks. It is scarcely applicable when a large number of samples need to be analyzed, because the available microwave rotors allocate only a few vessels in every digestion cycle. Moreover, it requires the use of concentrated acids with careful monitoring of digestion, and more than one cleaning cycle is necessary to avoid any memory effects from vessel walls. Procedures for sample preparation with minimal handling are much more desirable. In this sense, some strategies have been developed and applied, such using tetramethylammonium hydroxide (TMAH) and formic acid for solubilization as well as slurry sampling, and laser ablation. Batista et al. [2] digested 75 mg of brain, kidney, liver, and heart samples in only 1 mL of 50 % (v/v) TMAH solution at room temperature for 12 h.

The use of slurries reduces analysis time and risks of contamination by circumventing sample decomposition with wet or dry oxidation methods, minimizing losses due to volatilization of certain elements. Furthermore, the use of slurry allows simplification of sample pretreatment and improvement of detection limits [3, 4]. In this connection, in Argentina, Aranda et al. [5] employed aqua regia and antifoam to determine Hg in serum blood samples. In Brazil, Ferreira et al. [6] determined Zn and Cu by flame atomic absorption spectrometry (FAAS) in a standard reference material of human hair through slurry sampling with 2.0 mol L^{-1} nitric acid solution, achieving detection limits of 88.3 and 53.3 ng g^{-1}, respectively.

Laser ablation analysis offers the possibility of elucidation of the spatial distribution of trace metals, and in combination with inductively coupled plasma mass spectrometry (ICPMS) facilitates trace element determination using small amounts of samples without any elaborate sample preparation. Laser ablation features were exploited by a Chilean group [7] in collaboration with researchers from the USA for As determination in hair of 7,000-year-old mummies from the coastal region of the Atacama Desert in the northern outpost of present-day Chile. The drinking water in this region is rich in As, and the mummies were

Table 1 Biological matrices, elements determined, techniques employed, and Latin American country where the studies where conducted

Biological sample	Elements analyzed	Technique	Country	Reference
Whole blood Urine	Cd	ETAAS	Brazil	[8]
Whole blood Urine	Mn	ETAAS	Brazil	[9]
Whole blood Urine	Pb	ETAAS	Brazil	[11]
Urine	Hg	CV-AAS	Brazil	[12]
Brain Kidney Liver Heart	Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Pb, Sb, Se, Sr, V, Zn	ICPMS	Brazil	[2]
Human milk	Al, Cr, Mn, Fe, Ni, Cu, Zn, Se, Cd, Pb, Ba, Co, As	ICPMS	Brazil	[13]
Urinary calculi	Earth rare elements	ICPMS	Argentina	[14]
Human hair	Cr, Co, Ni, Cd, Pb	HR ICPMS	Brazil	[15]

CV-AAS cold vapor atomic absorption spectrometry, *ETAAS* electrothermal atomic absorption spectrometry, *HR* high resolution, *ICPMS* inductively coupled plasma mass spectrometry

found in As-endemic areas. Well-preserved mummy hair samples provided a unique opportunity to explore the ancient As exposure of the Chinchorros Indians (Chile).

Analytical techniques used for determination of metals and metalloids in biological samples

Atomic absorption spectrometry

Electrothermal atomic absorption spectrometry (ETAAS) has been shown to be especially attractive for the analysis of biological samples, since it requires low sample volume and provides adequate low detection limits. In Brazil, De Moreira et al. [8] employed a minimum sample pretreatment using a more elaborated furnace temperature program with chemical modifiers to achieve in situ matrix elimination prior to the atomization step. It was applied to the determination of Cd in certified reference materials (CRMs) of whole blood and urine. In the same country, Luna and De Campos [9] determined Mn in whole blood and urine by ETAAS using different modifiers, employing aqueous calibration with 100 % recoveries. The volatility of some compounds is a concern in the pyrolysis step in ETAAS determinations in complex samples [10]. In this connection, Grinberg and De Campos [11] introduced Ir as a permanent modifier and used a factorial design. The conditions selected made it possible to perform up to 1,100 firings with the same coating in the determination of Pb in whole blood and urine CRMs. In addition, Zenebon et al. [12] determined Hg in urine from workers exposed to Hg vapor by cold vapor atomic absorption spectrometry (CV-AAS), achieving a throughput of 40 samples per hour.

Plasma-based techniques

Plasma-based techniques are choice techniques for the analysis of this kind of matrices. In Brazil, Batista et al. [2] determined Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Pb, Sb, Se, Sr, V, and Zn in brain, kidney, liver, and heart samples after a simple sample digestion with TMAH. Nascimiento et al. [13] determined Al, Cr, Mn, Fe, Ni, Cu, Zn, Zn, Se, Cd, Pb, Ba, V, Co, and As in Brazilian human milk using aqueous calibration. The sequential multielemental measurement capability of ICPMS was also demonstrated by an Argentinean group [14] that determined 14 rare earth elements in urinary calculi by the standard addition method.

The usefulness of high-resolution ICPMS for these studies was demonstrated by researchers from Brazil [15] who reported a method to identify and quantify Cr, Co, Ni, Cd, and Pb in human hair samples that was virtually free from spectral interference.

Other elemental techniques

To a lesser extent, X-ray fluorescence and neutron activation analysis have also been used by groups in Argentina and Brazil in the development of methods for analysis of biological samples and even speciation approaches.

Preconcentration procedures used for elemental analysis

General considerations

In spite of the sensitivity and selectivity achieved by atomic spectrometric techniques, the concentration of the analytes of

interest are so low that, in many cases, their preconcentration before analysis is mandatory. For many years, liquid/liquid extraction has been the method of choice for analyte preconcentration and/or matrix removal. Nowadays, SPE has become a convenient alternative for both analyte preconcentration and matrix removal. The selection of the sorbent is of prime importance, and researchers in this field are devoted to the development of highly specific substrates. Sorbents with immobilized chelating reagents [16], dithizone [17], immobilized yeasts or amino acids in controlled-pore glass [18], and more recently, carbon nanotubes [19] have been used satisfactorily.

Although most of the work done has been concerned with separation of analytes from a troublesome matrix or with preconcentration, other authors have combined selective retention or selective elution for the selective determination of species [20].

Some selected articles on the use of preconcentration methods for elemental determination in biological matrices are included in this survey, and they are cited in Table 2.

Solid phase extraction

Different Brazilian groups have been pioneers in the development of preconcentration methods involving flow injection systems. One of the first substrates studied as a possible sorbent in online procedures was polyurethane foam (PUF). A PUF minicolumn was used for the first time in Latin America for the online preconcentration of Zn retained as a zinc thiocyanate complex [21] and determined by spectrophotometry using 4-(2-pyridylazo)resorcinol. A detection limit of 0.9 ng mL^{-1} and a relative standard deviation of 1.2 % were reported for a preconcentration time of 1 min.

Subsequently reported procedures introduced more selectivity for metal determination. To this end, Lemos et al. [22] filled a column with PUF-2-(2-benzothiazolylazo)-2-*p*-cresol that was inserted online in a flow system coupled to a FAAS system. A preconcentration factor of 41 (when 7 mL of sample was processed) and a detection limit of 0.27 μg L^{-1} were reported. The method was applied to the determination of Cd in CRMs (rice flour, bovine liver, and lobster hepatopancreas), with certified concentrations ranging from 0.31±0.02 to 25.45±0.70 μg g^{-1}. In a new approach, a procedure was described for the sorption of Cu(II) ions onto a minicolumn of PUF loaded with the chromogenic reagent 4-(2-pyridylazo)resorcinol and subsequent online determination by FAAS [23]. The method allowed determination of Cu(II) ions with a detection limit of 0.35 μg L^{-1} in water (mineral water and tap water) and high-salt aqueous samples [physiological serum containing 0.9 % (m/v) NaCl]. Some years later, a comprehensive review with 85 references of the main applications of PUF was provided by Lemos et al. [24].

A group from San Luis, Argentina, employed resins in different applications as follows. The coupling of flow injection with inductively coupled plasma optical emisssion spectroscopy (ICPOES) was studied for Bi preconcentration on an Amberlite XAD-7 column [25]. The detection limit for the preconcentration of 100 mL of aqueous solution was 30 ng L^{-1}. The measured Bi concentrations in real samples were between 0.76±0.04 and 5.21±0.02 μg L^{-1}. In another study, quinol-8-ol and Amberlite XAD-7 were used for Bi preconcentration and online determination by hydride generation ICPOES coupled with flow injection in real urine samples [26]. Bismuth concentrations ranged from 0.40±0.03 to 3.35±0.02 μg L^{-1}.

The resin Amberlite XAD-7 was also assayed for the preconcentration and determination of Co in human urine samples by ICPOES coupled with flow injection [27]. The analyte was retained on the resin as a cobalt-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complex. The high sensitivity of the method was demonstrated by an enhancement factor of 90, making it possible to achieve a limit of detection for Co of 25 ng L^{-1}. The concentrations of Co measured in real samples were in the range 0.27-0.52 mg L^{-1}.

In Brazil, the resin AG50W-X8 was employed in a microscale flow system for online preconcentration of Cd, Cu, Mn, Ni, and Pb in saliva samples and their determination by ICPOES [28]. A sample volume of 1 mL was required to obtain enrichment factors of 46, 23, 17, 18, and 44 for Cd, Cu, Mn, Ni, and Pb, respectively.

Another Brazilian group [29] reported an online preconcentration method for Au, Ag, Te, and U determination using solenoid valves and a minicolumn filled with C_{18} immobilized on silica to retain the complexes formed with the ammonium salt of *O*,*O*-diethyl dithiophosphoric acid. Detection limits ranged from 0.05 to 2.24 pg mL^{-1} for U and Te, respectively. The preconcentration factors ranged from 2.6 to 180 for U and Te, respectively.

Argentinean researchers employed activated carbon for preconcentration purposes. CV-AAS was used for the preconcentration of Hg on a column and subsequent determination in digested hair samples [30]. Optimization of both preconcentration and generation of Hg volatile species was conducted using a two-level full factorial design (2^3) with three replicates of the central point. The average Hg concentration in the samples under study was 210 ng g^{-1}.

Cloud point

In Argentina, the use of the cloud point constitutes a field of research especially in a group from the University of San Luis. It is a simple and low-cost method that can be easily

Table 2 Preconcentration methods and techniques adopted for elemental determination according to the country where the studies where conducted

Biological samples	Elements	Preconcentration method	Country	Reference
Biological CRMs	Zn	SPE	Brazil	[21]
Liver Hepatopancreas	Cd	SPE	Brazil	[22]
Serum	Cu	SPE	Brazil	[23]
Urine	Bi	SPE	Argentina	[25]
Urine	Bi	SPE	Argentina	[26]
Urine	Co	SPE	Argentina	[27]
Saliva	Cd, Cu, Mn, Ni, Pb	SPE	Brazil	[28]
Plasma	Au, Ag, Te, U	SPE	Brazil	[29]
Hair	Hg	SPE	Argentina	[30]
Urine	Dy	CPE	Argentina	[31]
Urine	Ga	CPE	Argentina	[32]
Saliva	Pb	CPE	Argentina	[33]
Urine	Hg	CPE	Argentina	[34]
Urine	Al	SPE[1]	Argentina	[35]
Saliva				
Hair				
Urine	Gd	Chemofiltration	Argentina	[36]
Urine	Gd	KR	Argentina	[37]
Serum	Fe, Pb	Coprecipitation	Brazil	[38]

CPE cloud point extraction, CRM certified reference material, *SPE* solid phase extraction, *KR* knotted reactor

combined with different analytical techniques, including the less sophisticated ones, which are generally available in routine and research laboratories.

Ortega et al. [31] reported an online Dy preconcentration system for determination of Dy in urine samples based on the combination of cloud point extraction and flow injection analysis and subsequent determination by ICPOES. A similar approach was used for Gd determination in urine samples [32]. The same group incorporated a cloud point extraction step prior to capillary electrophoresis for the simultaneously determination of Pt and Pd at submicrogram per liter levels in spiked water and urine samples.

In 2006, Luconi et al. [33] developed a method for Pb determination in saliva to detect potential fresh Pb exposures since saliva draws upon the circulating plasma, which is the first internal compartment in which absorbed Pb resides. They developed a micelle-mediated phase separation without chelating agents addition as a prior step to Pb determination by capillary zone electrophoresis. The same reaction medium was employed for Hg preconcentration, and a limit of detection of 16 μg L^{-1} was achieved [34].

Other strategies have been developed, such as the use of more specific and selective substrates. In this direction, use of L-methionine immobilized on controlled-pore glass for metal sorption was attempted by Pacheco et al. [35]. The sorbent tested was packed in a conical minicolumn and connected to a flow injection–ultrasonic nebulization–ICPOES system for Al determination in urine, hair, and saliva samples.

Chemofiltration

In Argentina, De Vito et al. [36] proposed an online Gd preconcentration and determination system coupled with flow injection and spectrophotometric detection. The online coupling led to a limit of detection of 15 μg L^{-1}.

Knotted reactor

Knotted reactors constitute an alternative for online preconcentration systems. Automatic preconcentration systems using solenoid valves have been proposed.

Salonia et al. [37] used a PTFE knotted reactor for Gd preconcentration. The system implemented with ultrasonic nebulization was coupled to a ICPOES system, associated with flow injection. The preconcentration system allowed an enhancement factor of 255 and a limit of detection of 4.0 ng L^{-1} to be achieved. Gadolinium concentrations found in real urine samples were between 0.32 and 0.50 μg L^{-1}.

Coprecipitation

Coprecipitation is another alternative that has scarcely been used for preconcentration purposes. Iron and Pb ions were coprecipitated using the violuric acid–Cu(II) system as a collector. The precipitate was dissolved and metals were determined by atomic absorption spectrometry [38]. The limit of detection was 0.18 μg L^{-1} for Fe and 0.16 μg L^{-1} for Pb.

Bioanalytical research on chromatographic and nonchromatographic methods applied to speciation analysis

Nonchromatographic speciation methods

In the past, the determination of total element concentration was considered to be enough for clinical studies. Although this is still useful in many areas, speciation analysis is of primary importance in bioanalytical studies mainly to identify metal species with adverse effects on living organisms. Another alternative is to distinguish between organic and inorganic compounds using simple and selective extraction or volatilization steps associated with simple instrumentation, reduced analysis time, low costs, and even with better accuracy than that obtained by chromatography-based methods.

Nonchromatographic speciation strategies are focused on a limited number of species and in most cases only discriminate groups of species as a function of their polar or nonpolar character, their molecular or ionic nature, their oxidation state, or their reactivity in the presence of a selected reagent. So, it is unlikely that nonchromatographic methods could provide information about all the species present in the sample and replace chromatographic methods. Table 3 shows some developments in nonchromatographic speciation approaches for biological samples conducted in Latin America.

Selective hydride generation

Derivatization by hydride generation and subsequent detection of the analyte in the gas phase by different techniques has become the most popular method for nonchromatographic trace element speciation. The group of Burguera and Burguera from Venezuela has conducted many studies devoted to the determination of toxic elements in biological matrices. Employing cold vapor methods, Gallignani et al. [39] have developed a method for the determination of total and inorganic Hg in urine samples by means of a time-based injection system used in conjunction with CV-AAS and microwave-aided heating. Martinis and Wuilloud [40] from Argentina reported a cold vapor ionic-liquid-assisted headspace single drop microextraction in conjunction with ETAAS that allowed a detection limit of 10 ng L^{-1} to be obtained for Hg. Recently, Lehmann et al. [41] described a hydride generation method using a metallic atomizer after microwave-assisted extraction for inorganic As speciation in biological samples.

Solid phase extraction for selective retention of species

The use of SPE associated with nonchromatographic speciation methods was also a topic of research in different Latin American laboratories. Selectivity was achieved by the functionalization of the sorbent by the immobilization of different types of molecules on its surface. The development of functionalized sorbents has been a field of research in Argentina in the last few years. Pacheco et al. [42] preconcentrated and selectively determined Sb(III) and Sb(V) in urine samples by employing L-methionine immobilized on controlled-pore glass. A fully automated online system, implemented with hydride generation and ICPOES led to an enrichment factor of 20 and a detection limit of 70 ng L^{-1} being obtained for Sb(III). Employing the same sorbent and ICPOES with ultrasonic nebulization, the same researchers determined selectively V(V) and V(IV) in urine [43]. A detection limit of 0.008 ng mL^{-1} and an enhancement factor as high as 900 were reported.

Cloud point extraction

Cloud point extraction was also used for speciation purposes. Oliveira Souza and Tarley [44] determined Sb(III) and Sb(V) in blood serum. Optimization was performed by a multivariate design, and a high preconcentration factor of 229 was obtained.

Nonchromatographic strategies can be used as fast and cheap alternatives to the chromatography-based speciation. On the basis of the knowledge provided by the aforementioned procedures, it could be possible to improve regulations concerning maximum tolerable levels of trace element species in different matrices and to open up new perspectives in clinical diagnostics, being possible to use nonchromatographic strategies as screening or specific tools as a function of the species and samples considered.

Bioanalytical research on chromatographic speciation methods

Research toward understanding the mechanisms of interactions of trace elements and metal probes with bioligands requires analytical techniques able to provide information on the identity and concentrations of elemental species occurring in biological tissues at picogram and even lower levels. The acquisition of data of this type is possible with "hyphenated" (coupled) techniques that combine a high-resolution separation technique with sensitive element-specific or molecule-specific detection. This section will be focused on the hyphenation of separation techniques to elemental detectors applied to speciation analysis in biological samples in Latin America. Table 4 summarizes the work done to this end.

Few articles from Latin America have reported on chromatographic speciation analysis applied to

Table 3 Chemical species and techniques adopted for speciation analysis according to the country where the studies were conducted

Biological sample	Species	Technique	Country	Reference
Urine	Total Hg Inorganic Hg	Selective HG	Venezuela	[39]
Hair	Total Hg Inorganic Hg	Selective HG[1]	Argentina	[40]
Urine	Sb(III) Sb(V)	SPE	Argentina	[42]
Urine	V(IV) V(V)	SPE	Argentina	[43]
Blood Serum	Sb(III) Sb(V)	CPE	Brazil	[44]

HG hydride generation

biological samples. However, some tendencies can be observed. Arsenic is a topic of permanent interest in Latin America because of its natural occurrence in waters in many countries, and consequently this element has been studied by several authors. In Chile, Yáñez et al. [45] studied four As species, including As(III), dimethylarsinic acid, monomethylarsonic acid, and As(V) in hair samples, and correlated the concentrations found with As levels in water sources. They found that As(III) in hair shows the best correlation with chronic exposure to As(V) in comparison with other species and total As. Two groups from Argentina in collaborative work determined different phenylarsonic compound species in urine, such as roxarsone, *p*-arsanilic acid, and nitarsone [46]. These compounds are used as additives to chicken and swine feed. Dórea et al. [47] determined methylmercury and ethylmercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. Young children are highly vulnerable to Hg insults because of highly diminished metabolism, sequestration, and excretion [48]. Another group from Chile [49] determined Sb(V), Sb(III), and $(CH_3)_3SbCl_2$ in urine of exposed individuals living close to an smelter–electrorefinery plant. Only Sb(V) was present in the urine samples analyzed. The presence of Sb(V) in urine could involve either direct exposure to pentavalent Sb compounds or exposure to Sb(III) and its further oxidation to Sb(V); however, the oxidation reactions have not been well documented in human fluids. In contrast, pentavalent antimonial compounds have clinical applications in the treatment of leishmaniasis, and many studies have been conducted in countries affected by this illness. In Brazil, Miekeley et al. [50] studied Sb metabolism in biological samples from patients treated for leishmaniasis.

Atomic fluorescence spectroscopy shows a great advantage in comparison with ICPMS because it can tolerate the introduction of solutions with higher contents of organic solvents. In addition, atomic fluorescence spectroscopy is less expensive, with comparable detection limits, and for this reason it was adopted by laboratories that were not able to purchase a more costly instrument. This technique has been successfully applied to determination of As, Hg, and Sb species in different biological samples.

Summary and perspectives

For many years Latin American researchers have escorted the different advances in Analytical Chemistry toward the determination of key elements in biological samples. In many cases, especially in the past, comparable results have been achieved with simple, less expensive, and less sophisticated techniques that were not available in many laboratories. Nowadays, more and more instruments are installed every year in different research centers and universities. In spite of these advances, only a limited number of countries are involved in the determination and speciation of key elements in biological samples: Argentina, Brazil, Chile, Mexico, and Venezuela.

Table 4 "Hyphenated" techniques applied to the speciation analysis of key elements in biological samples

Biological samples	Species	Technique	Country	Reference
Hair	As(V), As(III), monomethylarsonic acid, dimethylarsinic acid	LC–ICPMS	Chile	[45]
Urine	Roxarsone, *p*-arsanilic acid, nitarsone	LC–AFS	Argentina	[46]
Hair	Methylmercury, ethylmercury,	LC–AFS	Brazil	[47]
Urine	Sb(III), Sb(V), $(CH_3)_3SbCl_2$	LC–AFS	Chile	[49]

LC liquid chromatography

In spite of the drawbacks, Latin American researchers have been pioneers in some specific areas such as the development of preconcentration methods.

Fortunately, the landscape described above has changed in the last few years. Nowadays, advances in speciation analysis and the emerging studies in metallomics show continuous and irreversible progress.

Research in Latin American has emerged and consolidated thanks to the continuous efforts of research groups as well as the increasing support of national research agencies, governments, and private companies that has allowed researchers to realize the dream to be now part of the international scientific community producing good-level science.

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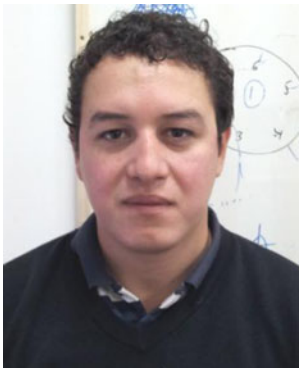

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