Review

Determination and speciation of metals by liquid chromatography

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Abstract

The development of liquid chromatography (LC) separation techniques for the determination and speciation of metal ions are reviewed over the last six years with particular regard to ion chromatography and complexation ion chromatography. Attention has been devoted to all LC applications of metal speciation studies and to their potential, when coupled to atomic or mass spectrometric detection systems. Examples are given for different mechanisms and materials involved as well as for developments in detection technology. © 1997 Elsevier Science B.V.

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1. Introduction

Metal ion determination and speciation by liquid chromatography (LC) has been reviewed [1] with particular regard to ion chromatography (IC) [2] and complexation IC [3].

This review focuses on the development of LC separation techniques for the determination and speciation of metal ions over the last six years. Particular emphasis has been given to the numerous
applications which use EDTA as a ligand with the above described techniques. The lanthanide metal ions display a relatively steady variation of properties across the group, as a result of lanthanide contraction, and pronounced chemical similarity. This last property makes it difficult to separate rare earth elements (REEs) and they have been considered as a unique independent family of analytes. The environmental and toxicological effects of a metal often depend on its forms. The determination of the total metal content does not provide this information.

Reversed-phase high-performance liquid chromatography (RP-HPLC) is quite suitable for this purpose. In fact it allows one to separate non-polar and moderately polar species of the same analyte while more polar and ionic species have been separated utilizing secondary equilibria such as ion suppression, ion-pair and ion-exchange. Several detectors including electrochemical detection (ED) inductively coupled plasma (ICP) and graphite furnace atomic absorption (GFAA) have been used for HPLC and studies have also been devoted to inductively coupled plasma-mass spectrometry (ICP-MS) coupled to LC [4,5]. Arsenic, selenium, lead, mercury, tin and chromium have been the subject of the majority of studies involving element specific detection for HPLC and IC. Examples are provided for different mechanisms and materials involved as well as for developments in detection technology.

2. Reversed-phase chromatography

A system that has received a great deal of attention for the analysis for trace metals is the formation of metal chelates with separation by RP-LC on C₁₈ columns and use of organic-based mobile phases. Dithiocarbamates are the most frequently reported complexing agents due to the strong chelating ability of their sulphur groups and their ability to form nearly water-insoluble metal salts with all metals except sodium and other alkali and alkali earth metals. Dilli et al. [6] completed a comprehensive study on RP-HPLC behaviour of diethyldithiocarbamate (DEDTC) complexes of Cu, Co, Cr, Ni and Hg with a variety of columns and mobile phases. In this case DEDTC complexes were pre-formed off-column (60°C, 15 min), extracted into chloroform and finally dissolved in CH₂OH and injected for the separation onto a C₁₈ column (μBondapak, Waters). The study showed that the ligand must also be present in the mobile phase for low concentration of chelates, to avoid their dissociation. In addition it has been demonstrated that the most suitable eluent is water–methanol rather than a methanol–acetonitrile–water mixture. In this way one avoids interferences due to the involvement of acetonitrile in ternary complexes formation. A similar procedure was developed for Co and Ni DEDTC–chelates separation on dimethyloctadecylsilyl-bonded amorphous silica. The preconcentration of chelates by a double extraction (diethyl ether followed by methanol) coupled with UV detection allowed González Rodríguez et al. [7] to reach 5 and 50 μg/l detection limits for Co and Ni, respectively. On-column DEDTC chelates formation–preconcentration and RP-HPLC separation has been carried out for the determination of Cd, Cu and Ni in sea water samples [8]. A C₂-bonded silica microcolumn, loaded with a dithiocarbamate–cetyltrimethylammonium ion pair, enabled the retention of metal ions and their complexation products. Elution on ODS analytical columns was optimized by adding cetyltrimethylammonium (CTA) bromide to a CH₂CN–water mixture, owing to the relative instability of Cd–DEDTC anionic complex which was eluted as a neutral ion pair. To overcome the main drawback of an extraction step, due to the low solubility of DEDTC complexes, a thiosemicarbazone was synthesized capable of forming soluble complexes [9]. 2-Acetylpyridine-4-ethyl-3-thiosemicarbazone complexes of Co, Cd, Fe, Ga, Ni, In and Zn were separated on a poly(styrene–divinylbenzene) (PS–DVB) column using a mixed-mode [RP and ion interaction chromatography (IIC)] mechanism since Co, Fe and In complexes are positively charged and the best separation was achieved by adding NaClO₄ to the CH₂CN–water mixture. The problem arising from the presence of more than one coordination form for the corresponding central ion for nitrogen–oxygen coordinated complexes (which produces a general peak broadening) has been tentatively solved by Ming et al. [10]. Their work concerns a pre-column and on-column derivatization for the separation of V(V), Co, Fe and Ni through the formation of binary and ternary peroxo complexes with 4-(2-
pyridylazo)-resorcinol (PAR) and $\text{H}_2\text{O}_2$. The study showed that, with methanol–water eluent and a RP column, $\text{V(V)}$–PAR binary complexes originate two peaks which are converted into a $V$–PAR–peroxo complex single peak if $\text{H}_2\text{O}_2$ is added to the sample within PAR before elution. 2-(5-Bromopyridylazo)-5-diethylaminophenol (5-Br-PADAP) is another highly sensitive and selective 2-pyridylazo complexing reagent. Studies for the separation and determination of metal ions [Cu, Co, Fe, Ni, $\text{V(V)}$, Pd] as 5-Br-PADAP chelates by RP-HPLC showed that only the retention of Co(III)–5-Br-PADAP complex is affected by varying the concentration of surfactant added to the eluent [11]. In addition a stronger interaction resulted for TBA with respect to CTA and CP. Alkyl groups, such as those of CTA or TBA, may interact with the $C_{18}$ chain on the stationary phase by molecular interaction so that the charged part of surfactant is exposed on the surface increasing its polarity and so eluting the Co chelate earlier. Following this approach a selective preconcentration method with a cation exchange resin for RP-HPLC of the Co–5-Br-PADAP complex was more recently developed [12]. Co complex, in aqueous solution, is readily oxidized to the Co(III)–5-Br-PADAP cationic complex which is retained on a sulphonated XAD-4 resin. The Co is spectrophotometrically detected (588 nm) after elution onto a $C_{18}$ analytical column (Capcell SG-120) with a methanol–water eluent added with EDTA and TBA and without 5-Br-PADAP. The absence of 5-Br-PADAP favours the dissociation e.g., of Cu and Zn chelates and other metal ions eluted later, like Fe and Ni, do not interfere. The detection limit for Co in water samples is reported to be 5.9 ng/l.

Various azo dyes have also been considered for the chromatographic separation of metal chelates on an RP-18 column and the study was focused on the separation and determination of $V(V)$ at trace levels [13]. The originality of this investigation is due to the optimization of the RP column selectivity by introducing a tetraalkylammonium salt into the system. The considered metal chelates are neutral or cationic and ion-paired complexes are not involved whereas other metal ions (e.g., Fe, Al) do not interfere in the determination. 8-Quinolinol (HQ) is another extensively used ligand for the separation of metal ions by HPLC. For this ligand methods are based on metal ion complexation, usually by heating the sample in the presence of HQ, one- or two-step extraction with compatible eluent solvent and injection of complexes into the chromatographic system [14]. To overcome the problems arising from off-line complexation and preconcentration (e.g., sample poisoning, time consuming) a column-switching technique has been proposed [15]. The term “column-switching” includes all techniques in which the direction of the flow of the mobile phase is changed by valves so that the effluent from the primary column is passed to a secondary column for a defined period of time. In the mentioned study [15] two compatible eluents of different eluotropic strengths were selected, one (CH$_3$CN–water) to concentrate the metal–HQ complexes onto a pre-column (Nucleosil $C_{18}$) and the second (CH$_3$CN–water–HQ) to elute the analytes from the pre-column onto the analytical column ($C_{18}$). The linear dynamic range is from 5 ppb to 10 ppm for Al and from 40 ppb to 5 ppm for Cu and Fe.

LC has been shown to be a powerful technique for the determination of trace noble metals, and among the different ligands employed, thiazolylazo reagents play a remarkable role in RP-HPLC. Studies on the retention of Pd, Pt, Rh and Ru chelates of 1-(2-thiazolylazo)-2-naphthol (TAN) enabled the determination of Pd and Ru [16]. Basova et al. [17] separated the 4-(2-thiazolylazo)resorcinol (TAR) complexes of Rh, Ru, Cu and Co on an ODS column with acetonitrile. Separation and determination of Rh, Ru and Os chelates with TAR was also reported [18]. More satisfactory results have been obtained by using a new thiazolylazo reagent namely 2-(6-methyl-2-benzothiazolylazo)-5-diethylaminophenol (MBTAE) [19]. MBTAE complexes (Pt, Os, Ni, Co and Rh, Pd or Ir, Ru) were separated and determined by RP-HPLC using both $C_{18}$ and $C_8$ columns with methanol–$n$-butanol–water or methanol–water eluents, respectively [20,21]. The mechanisms involved in LC separation are often coupled to optimize sample composition, matrix removal and/or to achieve the separation of groups of metals through the different behaviour of their complexes. As an example of this approach the method for Th and U determination developed by Hao et al. [22] must be mentioned. An on-line matrix elimination of phosphate and other anions, after phosphate mineral
dissolution, is obtained by cation-exchange of nitrophosphate samples. Th and U retained into a cation exchanger are eluted within other cations into a C₁₈ pre-column with a mandelate eluent, where Th and U are retained while the lanthanides and transition metals are flushed directly to waste. Finally Th and U were transferred and separated into a C₁₈ reversed-phase analytical column as α-hydroxyisobutyric acid (HIBA) complexes with the HIBA eluent. The method gave linear calibration plots over the range 0–1.2 mg/l for both elements.

3. Ion interaction chromatography

Soap chromatography, ion-pair chromatography, dynamic ion-exchange chromatography and IIC are different names for a chromatographic process using eluents containing an ion interaction reagent (IIR). Stationary phases (conventional RP or polymers) are dynamically modified into low-capacity ion exchangers. Without detailing the mechanisms involved during the separation, the results obtained indicate that the retention of neutral analytes, analytes having the same or opposite charge with respect to the IIR, will be unaffected, decreased or increased. Elution of cations is achieved by their complexation with the eluent ligand and ion-pairing of negatively charged complex formed with IIR or their cation exchange with the counter-ion of IIR. So the research in this field is devoted to evaluating the nature and concentration of proper ligands and IIR as well as organic modifier and eluent pH. The pH of the mobile phase affects the retention and resolution of chelates since it can alter their stoichiometry and overall charge. Hoshino et al. [23] examined 2,2'-dihydroxyazobenzene and five substituted analogues as pre-column chelating agents for IIC of Al, Co, Cu, Fe, Mo, V and Zn with spectrophotometric detection. Two packing materials having different polarity [octadecyl silica (ODS) and propylciano-bonded silicas (CN)] in combination with buffered aqueous methanol, acetonitrile or tetrahydrofuran as the mobile phase, were tested, at different pH values. To mask metal contamination from the HPLC system a mobile phase containing 0.1 mM Na₂EDTA was used, metal chelates have been prepared off-line and TBA was employed as ion-pair reagent. One of the most interesting results was that azo chelates of typically labile ions such as Cu, Zn, V and Mo showed enough stability to be eluted. Starting from this study a method was developed for the determination of Al in human serum. The separation of Al–2,2'-dihydroxyazobenzene chelate was obtained on a C₁₈ bonded silica packing with an aqueous methanol mobile phase containing TBA; the UV detection gave a detection limit of 6 µg/l for the real sample [24]. A broad work on LC studies of metal complexes of nitroso-naphthol sulphonates ion-paired, with liquid–liquid extraction and on-line derivatization was carried out by Sirén [25]. A modification of the proposed methods consists of an on-line derivatisation of metal ions; in this procedure the metals are injected into a methanol–water eluent containing a quaternary ammonium bromide (CTA or TDTMA) and, after the column, they are mixed with a ligand solution (1-nitroso-2-naphthol-6-sulphonate) [26]. This procedure makes the metal ion separation governed by the kinetic of formation of complexes and ion pairs and retention onto the post-column mixer-reactor system. The chromatographic behaviour of 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxy-naphthalene-2,7-disulphonic acid (Plasmodin B) and its metal ions complexes has been studied in ion-pairing reactions for metal preconcentration and separation by HPLC [27]. The separation of analytes was optimized with a flow-gradient elution and the method, successfully applied to river water samples, enabled analyte metals to be separated from alkaline and alkaline earth elements. The detection limit of the whole procedure was lowered by addition of a preconcentration step. It was performed by eluting samples through an hydrophobic microcolumn (C₁₈) after the ion-pair reaction was carried out directly on the sample. The procedure gave enrichment factors up to 900 within detection limits between 15–90 ng/l [28]. In a similar way an ion-pair HPLC method for the determination of Cr(VI) and W(VI) has been developed based on the formation of ternary complexes by rutin (quercetin-3β-rutinoside) with Cr(VI) and W(VI) in the presence of CTA. The complexes were extracted on a solid-phase and recovered with methanol, although detection limits are not given, the method seems suitable for geological samples with a sensitivity of 5 µg/l for Cr(VI) [29]. A feature of IIC is the high
selectivity obtainable by the proper choice of ligand and chromatographic parameters. The method developed for Fe(II) determination in aerosol, rainwater and seawater (remote marine aerosol) is an example [30]. A solid-phase extraction (SPE) on Sep-Pak C_{18} cartridges loaded with 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-\(p,p'\)-disulphonic acid [ferrozine (FZ)] enabled the separation from matrix and preconcentration of Fe(II) as Fe(II)–FZ cation complex. The complex and FZ were eluted with methanol and excess ligand was separated by IP-RP-HPLC. In this way Fe(III), Ni(II), Co(II) and Cu(II) interferences were removed and a detection limit of 5.6 ng/l was obtained. Ohtsuka et al. [31,32] have separated several ion pairs of anionic metal chelates with pyridylazosulphoaminophenol derivatives (PAPS) on a C\(_{18}\) stationary phase. They elucidated the retention behaviour of metal chelates (PAPS) in IIC as a function of mobile phase composition [33] in respect to the significant differences found in methanol–water and acetonitrile–water systems as a function of the volume fraction of water [34].

Another approach to IIC is the use of common reversed-phase stationary phases permanently coated with suitable hydrophobic agents such as alkylsulphonates or alkylsulphates with a sufficiently long alkyl group. The mechanism of elution is governed by the mobile phase following two ways: (i) eluents containing strong driving cation and a small amount of complexing agent (“push–pull” method, e.g., mobile phase containing ethylene diammonium cation and tartaric acid), (ii) eluents containing a very weak driving cation and higher concentrations of complexing agent (“pure pull” mechanism). A more recent study on chromatographic behaviour of metal ions in IIC when the stationary phase is modified with various alkanesulphonates (1–10 carbon atoms in the alkyl chain) must be mentioned, the paper shows that a very good resolution is achieved even if in the ion-interaction mode the number of theoretical plates of the column is lower than that obtained in the reversed-phase mode [35].

Octadecyl-bonded silica permanently coated with sodium dodecyl sulphate (SDS) in the presence of complexing agents was considered for the separation of transition metals [36]. In that paper an ion-exchange mechanism similar to that of fixed sites exchangers was observed; both the pushing effect of the eluting cation and the pulling effect of the complexing anion are taking place but the pulling effect of the ligand plays a dominant role in the process of elution. A significant example, of the mentioned approach, is the detailed study of Cassidy and Sun [37]. They compared the performance of an anion separation with a cation separation both based on an ion-interaction system that used cetylpyridinium chloride or \(n\)-octanesulphonate to modify a reversed-phase. In the first case transition metals (Mn, Co, Ni, Cu and Zn) were eluted with an oxalate eluent. The anion-exchange system provided column efficiencies comparable to that for the cation system. This approach may be attractive for solving analytical problems taking into account the considerable different order of separation obtained by the two systems.

Reversed-phase ion-pair procedures involving EDTA have been considered in optimizing separation and detection of species. Different techniques like pre-column derivatization without complexing agent in the eluent or on-column derivatization may be less efficient and give rise to peak broadening.

The simultaneous determination of V(V) and V(IV) as complexes was achieved by using RP ion-pair chromatography [12% (v/v) CH\(_3\)CN in water, 50 mM TBA, 2.0 mM EDTA at pH 6] with conventional UV detection (50 μg/l detection limit), the method was successfully applied to the analysis of a leachate of waste catalyst [38]. IP-RP-HPLC has been investigated by coupling EDTA with tetra-ethyleammonium (TEA) [39], tetrabutylammonium (TPA) [39] and tetrabutylammonium (TBA) [39–41] bromide ion pairing agents. TBA was the most suitable ion pairing agent in all cases and the use of EDTA in the eluent [39,41], together with high complexation constants, shifted equilibrium in favour of chelates formation reaching lower detection limits. The data obtained [39] clarify some aspects of the separation mechanism of ion-interaction chromatography for different oxidation states metal ions and confirm that the retention of divalent and trivalent metal ions complexed with EDTA takes place through an ion exchange mechanism in which the ion exchanger is dynamically generated by the retention of the counterion in the stationary phase [41]. In addition to the separation of a mixture of Fe, Cr, Ni, Cu Mn, Pb and NO\(_3\), the evaluation of Cr speciation...
is performed and detection limits between 0.015 and 1.7 mg/l are achieved.

Theoretical approaches to the retention of anionic metal complexes have also been developed for IIC or dynamic ion exchange [42,43].

4. Ion chromatography

Metal ions can be retained on silica-based ion-exchange columns and silica itself can act as both anion- or cation-exchanger. Ion-exchangers produced by chemically bonding ion-exchange groups to a silica backbone were the main type of early column packings, pellicular ion-exchangers formed by coating a silica core with a polymeric ion-exchange material. Polymer-coated silica cation-exchangers for IC can be synthesized by depositing varying film thicknesses of a pre-polymer onto porous silica, immobilization is achieved by in situ cross-linking reactions using radical starters or radiation. Poly-(butadiene-maleic acid) (PDMA) is the preferred polymer. An advantage of silica-based materials is the low probability of secondary interactions between solute ions and the silica substrate and higher column capacities over synthetic material. On the other hand, serious drawbacks exist with silica-based materials. Both low and high pH values must be avoided, below pH 2 covalent bonds linking the ion-exchange functionality become unstable and the functional groups are cleaved, while over pH 8 silica matrix may be dissolved. A great improvement on metal ion chromatography was obtained with new synthetic packing materials. Basically this kind of resin is characterized by an ethyl vinylbenzene substrate cross-linked with 55% divinylbenzene surrounded by solvent compatible functional groups (sulphonic, carboxylic/phosphonic, carboxylic/phosphonic/crown ether) (IonPac Columns, Dionex, Sunnyvale, CA, USA).

The main difficulty which had to be overcome was the vastly different selectivity of the ion-exchange resins, normally used to separate monovalent and divalent cations, respectively. These resins were characterized by a much larger selectivity for divalent cations than for monovalent cations, thus divalent cations have greater retention times than monovalent. Column-switching, in addition to eluent-step change, coupled with different cation exchange columns, have also been proposed to solve this problem and the procedure was applied to seawater analysis [44]. More recently alkali and alkali earth elements have been successfully separated by isocratic single chromatography [45,46]. As an example cation-exchange separations must be mentioned based on citric and pyridine-2,6-dicarboxylic acids (PDCAs) or tartaric and dipicolinic acid mixtures as eluents [47,48]. A preconcentration procedure, obtained on replacing the sample loop with a cation-exchange microcolumn (CG12, Dionex), has been coupled with a cation-exchange analytical column and a methanesulphonic acid eluent [49]. This method enabled the ion chromatographic determination of Na⁺, K⁺, Mg²⁺ and Ca²⁺ in snow layers using only a 2 m sample volume. Dumont and Fritz [50] showed that increase in the separation factors and change in the elution order of alkali-metal cations could be obtained using non-aqueous solvents with macroporous cation-exchange resins (low-capacity PS-DVB sulphonated resin). This behaviour is due to the change in solvated ionic radii which largely affects Li and Na, so that a higher resolution is obtained in the separation of ions that usually elute close together (Li/Na, K/NH₄⁺). The use of lithium ion and neutral pH values or methanol and lithium chloride as the eluent gave very low detection sensitivities and poor separation of mono- and divalent cations on porous silica [51–53]. More recently the separation of six inorganic monovalent cations was achieved on a porous silica gel microcolumn with a 30% (v/v) acetonitrile and benzyltrimethylammonium (BTMA) chloride in the mobile phase [54]. This result could be explained through a reduction of the hydrophobic interaction between cations and BETMA introduced onto the surface of silica through cation-exchange and hydrophobic interactions. The advantage of the method is that BTMA cation both competes with analytes for the exchange sites and is used for their indirect UV detection. Cation-exchange on silica performed using weakly or neutral eluents gives very good sensitivities for cations. Ohta et al. [55] studied the cation-exchange properties of silica gel in the acidic region. They obtained the simultaneous separation and determination of Na⁺, NH₄⁺, K⁺, Mg²⁺ and Ca²⁺ in environmental water samples by coupling
nitric acid with a selective complexation PDCA of divalent cations. A synthesized weak cation exchanger (copolymerization of vinyl groups covalently bound onto silica surfaces with acrylic acid) has been used as stationary phase and the pure ion-exchange mechanism is modified by adding 18-crown-6 ether and acetonitrile to the mobile phase [56] for alkali and alkali earth cations separation. Among these, greatest efficiencies have been obtained with commercially available resins namely IonPac (Dionex). A complete separation of monovalent and divalent cations is achieved in less than 10 min by isocratic separation with e.g., sulphuric, hydrochloric, and methanesulphonic acid eluents. Some interesting applications of samples covering a wide range of concentration are summarised by the determination of elements in Antarctica samples [57,58]. The high capacity, which characterises some of these stationary phases, provided the means to develop a method based on high-volume direct-injection for trace level determination [59].

The above mentioned polymers in some cases (e.g., IonPac CS5) bear both quaternary ammonium and sulphonate functional groups, in a pellicular layer located on the core of the beads. The presence of both cation- and anion-exchange groups enables more sophisticated separations: (i) Sun et al. [60] speculated on this property and separated Ge and Sn, by pure ion-exchange, as cation and anion, respectively. Metals were determined simultaneously on a IonPac CS5 by working at the proper pH (<1.5), ensuring the presence of HGeO$_3^{-}$ and Sn$^{2+}$ species; (ii) Motellier and Pitsch [61] developed a LC method with an on-line preconcentration step (IDA chelating column) using PDCA which forms negatively charged complexes with metals (Co, Cu, Ni, Mn, Zn) and a CS5 analytical column. They showed that the exchange mechanism for Ca on the analytical column is much different from that of transition metals (pure ion exchange of positively charged complexes). Cu is in free form and reacts with PDCA in the eluent giving interference with other metals, the method proved to be suitable for waters of low ionic strength. A good comparison of chromatographic behaviour obtained with different mechanisms of separation could be made by comparing these results [61] with a pure cation-exchange separation for the same metals [62]. Better peak resolution was obtained with the separation obtained on the Dionex Ion Pac CS2 column (cation-exchange capacity 60 µeq) eluting with a mixture of ligands (citric and oxalic acid) than that made on the Dionex Ion Pac CS5 column (anion-exchange capacity 70 µeq) where probably not only the exchange but also interactions of complexes with the column matrix are acting.

In the cation-exchange technique the metal ions are normally reacted with an anion of a weak acid to reduce their charge density in the eluent solution before entering the separation column, where they are separated owing to their respective affinities towards the active sites of the separating resin. Ligands are also required to avoid precipitation when an acidic eluent is not suitable for the selected columns. A low-capacity silica-based cation-exchange column was used for the separation of transition metals (Co, Cu, Fe) and to enhance sensitivity coupled with post-column chemiluminescence detection [63]. In this case attention has been paid for the eluent composition not only to improve the separation but for its compatibility with the post-column reaction.

IC separation of metal ions based on anionic exchange offers the potential of different selectivity, reduced problems for metal ion hydrolysis and application to complex sample matrices. Notwithstanding many organic acids from mono-, di-, tri-carboxylic acids to chelating agents such as iso- butyric acid (HIBA), tartaric, citric, oxalic, PDCA, 1,2-diaminocyclohexanetetraacetic acid (DCTA), ethylene glycol bis(2-aminoethyl ether) tetraacetic acid (EGTA), diethylenetriaminopentaacetic acid (DTPA) have been evaluated for simultaneous IC of anions, alkali, alkaline earth and heavy metals [64,65]. EDTA plays a fundamental role. EDTA has also been used as a masking agent, to avoid metal ion interference arising from possible precipitation at the eluent pH. Since EDTA forms, at the proper pH values, negatively charged complexes with divalent or trivalent metal ions, the possibility of simultaneous separation of anions from metal ions is also feasible as well as the speciation of metal ions. In these procedures complexes can be obtained in two ways: the first way is through its formation before the chromatographic separation (pre-column complexation, complexes must be stable enough to avoid
decomposition during separation or ligand must be added to the eluent); the second way is based on the complexation in the chromatographic column itself. Some examples of applications of an EDTA eluent have been reported, for the determination of anions and divalent cations in natural and pharmaceutical samples at pH of 6–8 with both UV and conductivity detection [66]. Experiments were also performed with binary eluent systems comprising EDTA, as complexing agent. An UV absorption reagent was used to enhance detection limits for Ca$^{2+}$ and Mg$^{2+}$ [67,68]. For seawater samples [69], silica based anion-exchange analytical columns enhanced sensitivity and enabled detection limits from 20 µg/l for Mg$^{2+}$ to 0.4 mg/l for Ca$^{2+}$ with UV and conductivity detection and eluent pH at 4.8. Komarova et al. [70] studied the ion chromatographic behaviour of anionic EDTA complexes of vanadium (V) and (IV). At higher pH required to elute the anionic complexes, [VO$_2$Y]$^{3-}$ complex could be decomposed and the high acidity in suppressor column originates [VO$_2$H$_2$Y]$^{5-}$, [VO$_2$H$_3$Y]$^{5-}$ and [VO$_2$H$_4$Y]$^{5-}$ species.

As mentioned above the IC determination of metal–EDTA complexes can be performed with anion-exchange columns [66,69,71–74] and anions and metals can be separated as anionic complexes in the same run. In addition by evaluating the chromatographic behaviour of single complexes this approach also enables specific determinations, e.g., Se(VI) was determined (detection limit 4.8 µg/l) in real samples by suppressed anion-chromatography in the presence of anions and heavy metal ions [72]. Alternatively the separation of the metal–EDTA complexes can be carried out with a cation-exchange column [65] in this case the retention mechanism of analytes involves the cation-exchange of free metal ions (e.g., Cu, Fe, Zn, Ni, Pb, Mn, alkali and alkaline earth metals) which are present at low pH values. Theoretical approaches to the retention of anionic metal complexes have been developed for anion-exchange [73–75] and for cation-exchange [42,76]. Detailed studies on the metal–EDTA complexes behaviour in cation chromatography as a function e.g., of pH and ionic strength, provides data that can be used to develop selective models for understanding the contribute of different charged or neutral species on the retention mechanism involved. A selective method for preconcentrating and determining Pb at trace levels, 0.5 µg/l detection limit, was developed based on these considerations [77].

5. Chelation ion chromatography

Trace metal analysis with ion chromatography procedures becomes very difficult with samples of high ionic strength such as concentrated brines or seawaters as the ion-exchange sites can become “swamped” with salt ions or in the case of ion-pair chromatography the pairing mechanism is modified. For these kinds of samples the selectivity of the chromatographic separation can be enhanced by the use of chelating chemically bonded phases where complexation reactions in the stationary phase, ion-exchange due to free or protonated chelating groups which act as ion-exchange sites, and in some cases complexation in the eluent, are responsible for the separation. Two main approaches can be followed to obtain proper stationary phases: to chemically bond the chelating group to the substrate, or to coat a substrate with a ligand which is permanently trapped onto the substrate. Kantipuly et al. [78] illustrated the extensive range of chemically bonded chelating resins available for the separation and concentration of trace metals, among many combinations of chelating ligands and supporting materials. Voloschik et al. [79] used a silica gel-based sorbent with chemically bound amidoxime functional groups for the selective determination in waters of metals (Mn, Pb, Cd, Co, Ni, Zn, Cu and Hg) since this kind of resin showed a weak affinity for Mg and Ca and this eliminates their interference in the determination. The separation of mono- and divalent-cations on a polybutadiene maleic acid (PDMA) stationary phase coated on silica materials has been performed with different eluents, mainly organic complexing ligands [80]. A detailed study on this material [81] enabled the determination of some transition metals by conductivity detection within detection limits from 35 to 410 µg/l for Cd and Pb, respectively.

The most widely used resin to preconcentrate and to separate elements and group elements in seawater, applied for the first time almost three decades ago [82], is an iminodiacetate resin (i.e., Chelex-100). The low degree of cross-linking of the microporous
PS—DVB supporting polymer made the Chelex-100 unsuitable for HPLC applications. Among many combinations of chelating ligands and supporting materials Bonn and coworkers [83,84] successfully characterized and used stationary phases with silica-bound iminodiacetic acid (IDA) functions. Due to the mixed mode-mechanism, ion-exchange and chelate formation, with eluents (e.g., citric or tartaric acid) the chromatographic behaviour of some elements (Mg, Fe, Co, Cd and Zn) is optimized but other ions (e.g., Cu, Ni and Pb) are irreversibly retained and strong complexing agents such as PDCA are required for the complete elution [85].

A more highly cross-linked macroporous PS—DVB containing the iminodiacetate functional group that allows operation at high pressure without physical degradation (MetPac CC-1 column, Dionex) has been developed [86]. At pH 5.2–5.6 polyvalent metal ions are selectively concentrated onto MetPac CC-1, alkali metals and anions are not retained and a selective elution of alkali earth metals could be achieved using ammonium acetate. Concentrated metals and lanthanides are eluted, with the exception of chromium, with acid onto a cation-exchange column, acting as interface before the analytical column, from which are successively driven to the ion chromatographic separation with a PDCA or an oxalate complexing eluent and detection accomplished after post-column derivatization (PAR). Detection limits (Fe, Cu, Ni, Zn, Co, Mn, Cd, Pb) range from 0.2 to 1 µg/l [86,87]. The procedure was successfully used for metal ions determination in seawater from the Venice lagoon and with 60 ml sample preconcentration the detection limits for Cu, Ni, Zn, Co and Mn were lowered to 0.05–0.1 µg/l [88]. A modification of the above mentioned procedure obtained metal recoveries from eluting resin with the same eluent used for the ion chromatographic separation (75 mM H₂SO₄–100 mM HCl–100 mM KCl) and a cation-exchange column with higher capacity. In this way for seawater samples (200 ml) detection limits of 10 and 30 ng/l are achieved for Cd and Pb, respectively [89].

The alternative approach to ligands chemically bound to silica or polymer phases refers to a technique applied from a long time in metal ions preconcentration and matrix removal before spectroscopic determinations based on permanent loading of sorbents by chelating agents [90–93]. Jones and coworkers widely investigated this field in order to improve detectability of both alkaline earth and heavy metals in brine samples. Using HPCIC the retention order of metal ions, including earth metals, is reversed with respect to IIC and so barium is eluted first as a sharp peak followed by Sr, Mg and Ca. The barium separation and determination at mg/l concentration in 1600 mg/l Ca samples with neutral hydrophobic resin (PS–DVB) firstly impregnated with Methylthymol Blue (3,3'-bis[N,N-di(carboxymethyl)aminomethyl]thymol-sulphonephthalein) coupled with an acid elution (0.5 M KNO₃+0.5 M lactic acid) and UV detection (PCR: PAR+ZnEDTA) [94] was successfully optimized showing a detection limit of 3 µg/l for Ba and Sr as well as Sr and Ca separation was optimized in milk powder analysis with phthalein purple (o-cresolphthalein-3,3'-bis-methyleneiminodiacetic acid) impregnated column [95]. Similar studies devoted to transition metal determination [96–98] resulted in an interesting procedure using a 10 µm particle size, 100 Å pore size PS–DVB resin impregnated with Xylenol Orange, stable from pH 0.5 to 11.5 [99]. The stationary phase enables Ca and Mg removal during the sample on-column preconcentration at pH 6 and the separation and determination of Zn, Pb, Ni and Cu with a step-gradient pH elution. The only drawback of the method is Cd–Mn coelution but satisfactory results are obtained for seawater samples (CASS-2 certified sample). This is a good example how small variations in chelating ability between dyes can be very useful allowing specific separations. A recent paper well details on investigation into the parameters involved in the production of a range of dye impregnated chelating columns (10 chelating dyes, mainly based on triphenylmethane or azo based dyes) for preconcentration and separation of alkaline earth, transition and heavy metals at trace levels within a devoted application to Al determination in seawater [100].

6. Rare earth elements separation

A separation of Y and Nd from Sm and the heavier lanthanides has been performed by low-
pressure liquid chromatography on a cation exchanger (AG 50W-X4) with a hydroxyethylenediaminetetraacetate (HEDTA) buffered eluent [101]; (i) their RP ion-pair separation, as a group, after chelation with DFB–Arsenazo [102]; (ii) a discussion on ion-exchange, both anion and cation and ion-interaction chromatography separation for La, Ce, Pr and Nb in magnesium alloys [103]; (iii) an ion-exchange chromatographic separation of rare earth elements in seven geological reference materials to remove matrix interferences in ICP-AES determination [104] as well as a review of their determination [105] could summarize the classic approaches to rare earth elements separation and determination.

The separation of rare earth elements by dynamic ion-exchange chromatography, on bonded phase silica (ODS) columns is strictly connected to the ion-interaction reagent (IIR) action. This could be performed in the following ways: (i) by a ligand, e.g., lactic acid (LA) or 2-hydroxy-2-methylpropionic acid (HIBA), isocratic or gradient elution with solution at constant pH and IIR, e.g., octanesulphonate (OS), concentration; (ii) by preloading ODS with an IIR, e.g., dodecyl sulphate (DDS), and both isocratic or stepwise acidity gradient elution of HIBA solution. The development of new resins enabled also the use of high-performance ion chromatography for lanthanides separation, better defined as chelating ion chromatography. Cation or mixed mode ion exchangers have been used with ligand gradient elution (e.g., HIBA+LA, oxalic acid+HIBA) and PCR–UV or ICP-MS detection.

Reversed-phase columns with both isocratic and gradient separation were coupled with ICP-MS detection. Isocratic conditions resulted in the rare earths separation into groups but was preferred for the powerful selectivity of ICP-MS, enabling 0.4–5.0 μg/l detection limits, and reducing analysis time [106]. Detection limits (DLs) obtained for analysis of geological materials were 1.0 mg/l for PCR–UV detection (post-column reagents: Arsenazo III [107,108], xylenol orange paired with cetylpyridinium bromide [109]) and 0.1–20 μg/l for the analysis of oxides by flame and inductively coupled plasma emission spectroscopy [110].

All lanthanide elements were separated in nitrophosphate fertilizer solution [111], magnesium metal alloys [112] and in less than 20 min with a good reproducibility in rock standard samples [113]. The method was applied to rock standards analysis accomplished by a group separation of rare earths by a preliminary treatment of acidic dissolved samples with a cation-exchange resin (Dowex 50W-X8) and 2 M HCl removing interfering matrix elements. Due to the appreciable overlap between Dy/Y peaks, and the concentration of Lu and Tm below the detection limits these elements could not be determined [113]. Conventional determination of lanthanide impurities in uranium requires large quantities of uranium and numerous isobaric overlaps due to β-decay and neutron capture restrict the direct determination of fission products and actinide isotopes by MS; therefore a chemical separation is required. A coupled-column chromatographic procedure based on a semi-preparative reversed-phase column and a cation-exchange column, has been developed [114]. The first column (ODS coupled with HIBA eluent) removes the uranium matrix and the analytical cation-exchange column, obtained by loading an ODS column with Caso4, enables lanthanide determination, after post-column reaction with Arsenazo III (0.02 μg/g U, detection limits). A cation-exchange chromatography and gradient elution with HIBA or LA enabled to overcome interferences due to polyatomic ions and isobars in ICP-MS detection of rare earths, e.g., GdO4 and GdOH+ overlap all the isotopes of Yb and Lu, and LaH+ hinders the free isotope of Ce, an IC–ICP-MS procedure has been developed. The method gave a complete separation of all rare earths within DLs of 1–5 ng/l and has been successfully applied to the analysis of Tm, Yb and Lu impurities in a Gd matrix and Ce in pure La2O3 [115]. Another example is the ICP-MS determination of fission product isotopes in irradiated uranium fuels where a chelation IC procedure [116] has been improved. U or U and Pu are preeluted with 1 M HCl or 0.4 M HNO3 and isobaric overlaps, present in direct mass spectrometric determinations, are removed as shown by measurements of isotopic composition of Nd in a high U and Pu matrix [117].

Kuroda and coworkers [118,119] showed that, in ion pair chromatography, glycolato complexes of rare earths are eluted within 20–30 min but their method suffers from incomplete resolution between Ho and Sm, Eu, Gd, Tb and Dy which coelute. The
same ligand was used in IC [120]. The first nine elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb and Dy) have been determined as impurities in a YbF₃ matrix, used in the production of optical fibers, in a more recent paper [121] by coupling oxalate and diglycololate as ligands in the eluent with an ion chromatographic system that requires a mixed mode ion-exchange packing.

Finally the separation of some lanthanides with chelating chromatography must be mentioned [122] where an HCl solution, instead of a chelating agent, was used as a mobile phase coupled with a selective chelating resin, namely a porous polymer bead impregnated with 2-ethylhexyl hydrogen 2-ethylhexyl phosphonate (PC-88A). The main advantages of this approach, in addition to high selectivity, are that sample solution does not contain concentrated salts or chelating agent and clogging of ICP torch and spectral interferences due to molecular bands are removed as well as the nebulization efficiency is optimized in respect to more viscous eluents. A similar approach based on selective retention of europium enabled the determination of trace impurities (aluminium, alkaline earth and seven transition metals) in high purity europium oxide [123].

7. Speciation studies

7.1. Arsenic

The molecular forms of arsenic subject to speciation analysis are anions, e.g., arsenite [As(III)], arsenate [As(V)], monomethylarsonate (MMA) dimethylarsinate (DMA) or cations e.g., arsenobetaine (AsB), arseneocholine (AsC) and tetramethylarsionium (TMA) ions or uncharged compounds at neutral pH e.g., arszenic acid.

A detailed study on AsB, AsC and TMAs cations was carried out by Blais et al. [124]. An evaluation of direct, ion pairing, reversed-phase, cation-exchange chromatographic procedures involving different stationary phases and eluents has been made in order to optimize the atomic absorption detection through a new on-line thermochemical hydride generation (THG) interface. Strong cation-exchange chromatography, solving the problem of AsC–TMAs coelution on C₁₈ column, was incompatible with the THG interface. The best choice resulted a normal-phase HPLC approach: cyanopropyl stationary phase and a methanolic eluent containing a silanol masking agent. The optimized procedure gave detection limits of 13.3, 14.5 and 7.6 ng for AsB, AsC and TMAs cations, respectively.

A simple method [125] has been developed for As(III) and As(V) speciation. An extractive chromatography has been performed by eluting samples (pH 2.5) through a stationary phase, silanized diatomite, modified with dioctyltin dichloride (C₈H₁₇)₂SnCl₂ [126]. As(V) was selectively retained and As(III) passed through the column, As(V) recoveries (85–115%) and column regeneration were obtained with 2 M HCl. The arsenic contents in the eluates were determined either by flame AAS or graphite furnace AAS, detection limits are not given but the linearity of the method was 0.01–0.20 μg/ml.

Arsenic speciation was evaluated, for As(III), As(V), AsB, MMA, DMA and p-aminophenylarsenate (p-APA) and AsC, by separation on an anion- and a CAS1 ion-exchange column and off-line hydride generation electrothermal atomic absorption spectroscopy [127]. In this study As(III) co-eluted with AsB. A thermal decomposition procedure to convert organoarsenicals into As(V) before reduction by sodium borohydride was introduced and detection limits resulted comparable to HPLC–HG-AAS [128] and HPLC–ICP-MS [129] procedures.

An anion HPLC procedure with a phosphate eluent was developed for As(III), As(V), monomethylarsonate, dimethylarsinate, arsenobetaine and arseneocholine separation [130]. After its optimization five peaks only were well resolved due to the overlap of As(III) and AsB. The analytes were determined by hydride generation atomic absorption spectrometry (HG-AAS). The arsenic content of the unresolved peak was assigned to As(III)+AsB or As(III), with or without a microwave-assisted oxidation step [131,132] before HG-AAS determination on two consecutive injections. The method applied to urine samples, with a clean-up procedure, gave detection limits within 8–15 μg/l and standard deviations of 2.5–5.3%.

A separation with a strong anion-exchange resin (BAX-10) and a gradient eluent concentration (K₂SO₄) at 60°C has also been proposed for As(III), As(V), MMA, DMA and AsB [133], but in this case
a complete separation was not achieved due to the coelution of inorganic species.

Rauret et al. [134] developed an IC–HPLC procedure for As(III), As(V), MMA and DMA separation and ICP-AES determination by coupling the systems with the hydride generation sample introduction technique. The procedure was improved [135] by checking two different kinds of column (Nucleosil-5SB and Hamilton PRP X-100) and by comparing isocratic and gradient elution. The peak profile was improved by filtering the data corresponding to low concentration with Fourier transform. With such a procedure detection limits between 2.7 As(III) and 11.4 As(V) µg/l were obtained.

Several reports on LC coupled with ICP-MS for metal speciation determination As have appeared [129,136–139]. A direct injection nebulizer interface work performed by Shum and coworkers [140,141] demonstrated that reversed-phase microbore columns and eluents containing ion-pairing agents could be coupled with MS detection for arsenic speciation. A good efficiency was also obtained using interfaces based on hydride generation manifolds. Hydride generation was tried to avoid the poor efficiency of conventional pneumatic nebulizers in LC–ICP-MS studies of arsenic speciation [142,143] but even by using a membrane gas separator [144] the determination was subject to the interference of ArCl⁺ molecular ion. A separation of As(III), As(V), MMA and DMA was performed with a reversed-phase ion-pair LC (IIIR: TBA–phosphate) [145]. The eluate was delivered to the hydride generation system after a prereduction with L-cysteine at 95°C in diluted nitric acid. The ArCl⁺ was removed and conventional detection limits were reduced to 11–51 ng/l.

Seven molecular forms of arsenic [As(III), As(V), MMA, DMA AsB, AsC and tetramethylarsonium (TMA)] were separated, by anion- and cation-exchange HPLC, and detected on-line by flame AAS. The potential interference due to phosphorus at 193.7 nm arsenic line is avoided because it is separated by anion-exchange HPLC procedure [139]. Silica based and polymeric cation-exchange columns were examined and a complete separation of cationic species and DMA was achieved by isocratic elution (pyridine eluent) on a bare silica column (~Si–OH groups act as cation-exchanging sites). The retention of DMA at low pH was attributed to the presence of DMA⁺ species. In a further development of the method, Larsen et al. [146] studied the determination of eight arsenic compounds in urine with anion- and cation-exchange separations by coupling HPLC–ICP-MS. The sample is silica C₁₈ cleaned and introduced into the system; four anionic [As(III), As(V), MMA and DMA] and four cationic [AsB, AsC, TMAs and trimethylarsine oxide (TMAO)] arsenic compounds were detected at m/z 75. Serious chloride interference due to polyatomic species ⁴⁰Ar ³⁵Cl⁺ which has the same m/z as the only arsenic isotope ⁷⁵As was overcome since chloride is separated chromatographically. In the cation-exchange chromatographic system the chloride eluted with the void volume and for the anion-exchange procedure eluted more than 100 s later than the last analyte peak well separated from arsenic species. TMAO, not included in the previous work, eluted in the cation-exchange system due to protonation of the As=O bond in the acidic mobile phase. The pH value of 10.3 for anion separation enabled the retention of arsenous acid which is eluted with the void volume in other anion-exchange chromatographic systems [147]. The procedure [146] has been applied to investigate the speciation of arsenic in seafood samples and elucidate the biosynthetic pathway involved in marine metabolism [148].

Caruso and coworkers [129,149] optimized the removal of ⁴⁰Ar ³⁵Cl⁺ interference by coupling ion chromatography and ICP-MS detectors and lowered detection limits for As(III), As(V), DMA and MMA with the use of an He–Ar gas mixture as ionization source: they ranged between 0.032 and 0.080 ng for DMA and MMA, respectively. More recently [150], a speciation of these compounds was obtained by micellar liquid chromatography coupled with ICP-MS detection. The method based on micellar mobile phase (CTAB, propanol and borate buffer), and on a PRP-1 separation column coupled with the ICP-MS system allowed linear dynamic ranges of three orders of magnitude and detection limits in the picogram range (90–300) and overcame the problems of chloride since it is not co-eluted with any of the four arsenic species.

A detailed study must be mentioned on suitability of the IS technique for arsenic speciation analysis in biological samples. A cation-exchange HPLC has been coupled to IS-MS(–MS) detection for analysis
of oragnoaarsenic species. Dual mode, elemental and molecular, analysis is presented using standard mixtures. Although detection limits are not low as those obtained by HPLC–ICP-MS the results indicate IS-MS–MS as complementary technique to ICP-MS for speciation analysis [151].

7.2. Selenium

The THG interface mentioned for arsenic speciation has also been used for HPLC–AAS determination of seleniocholine (SeC) and trimethylselenonium cations (TMSe) [152]. Cation-exchange chromatography was unsuitable for the THG interface and cations were separated on a cyanopropyl stationary phase with a methanolic phase containing a silanol masking agent. The calculated detection limits were 43.9 and 31.3 ng for SeC and TMSe, respectively. An improvement of chromatographic behaviour and reduced detection limits are observed by adding trimethylsulphonium iodide to the mobile phase. Houck et al. [153] obtained for inorganic selenium species, Se(IV) and Se(VI), detection limits of 10–20 ng/ml by ion-pairing reversed-phase (silica C18) microscale-liquid chromatography (eluent: methanol–water–TBA, flow-rate 50 μl/min) and a direct injection nebulizer (DIN) coupled with ICP-MS. More recently Yiang and Jiang [154] coupled ion-pair chromatographic separation, similar to the previous method, with ICP-MS detection using an ultrasonic nebulizer. Using this approach the detection limits for TMSe, Se(IV) and Se(VI) were 0.17, 0.76 and 0.53 ng/ml, respectively. Se(IV) and Se(VI) species were also separated by Shum and Houck [155] on an anion-exchange microcolumn (eluent: sodium carbonate–bicarbonate, flow-rate 100 μl/min) with a DIN-ICP-MS detection system. Isotope ratio measurements on chromatographically separated species of Se gave detection limits of 7–8 ng/ml for both of the species (based on 78Se and peak area measurements). A Nucleosil 100-SB anionic-exchanger column was used with an eluent ammonium citrate for HPLC fraction collection and electrothermal atomic absorption spectrometry of Se(IV), Se(VI) and TMSe speciation [156], the detection limits ranged from 11 to 32 ng/ml, in water and urine matrices. Inorganic selenium species in aqueous samples have also been separated using an anion-exchange column with a two-step eluent switching procedure [157]. The best separation was obtained with 25 mM K2SO4 eluent switching to 200 mM after 200 s at flow-rate 2.0 ml/min. After the separation by on-line acidification, microwave reduction and hydride generation the species were detected with an atomic fluorescence detector. The method provided detection limits of 0.2 and 0.3 ng/ml within a relative standard deviation (R.S.D.) of 1.5 and 2.0% for selenite and selenate, respectively.

Speciation of some organic selenium compounds has also been considered in a recent review [158]. Quijano et al. [159] reported the use of a mixed column for the speciation of selenocystine (SeCy), selenomethionine (SeMet), selenite and selenate with ICP-MS. The speciation of organic selenium compounds (SeCy, SeMet and TMSe ion) by HPLC–ICP-MS in natural samples (enriched yeast, human serum and urine) has also been performed on a reversed-phase analytical column (Hamilton RP1) [160]. By optimizing the eluent polarity and ion-pairing agent (pentane sulphonate) with regards to TMSe cationic species a satisfactory separation is achieved and inorganic species are eluted in the void volume. Detection was performed by ICP-MS using 82Se for quantification, and enabled to detect 0.20, 0.60 and 0.20 ng/ml for SeCy, SeMet and TMSe, respectively within a 0–500 ng/ml analytical dynamic range.

A detailed study on inorganic selenium and selenoaminoacids speciation has been made by on-line coupling a HPLC–microwave-digestion system with an AA, an ICP or an ICP-MS spectrometer [161]. The system proposed allows complete separation of selenoaminoacids in urine samples but the inorganic selenium peaks are overlapped, their speciation is however obtained by carrying out a second injection and detection with the microwave turned off. The ICP-MS detector provided the lowest detection limits, i.e., 0.16, 0.59, 0.66 and 0.19 ng/ml for total inorganic Se, selenomethionine, selenoethionine and Se(IV), respectively.

7.3. Lead

Organolead compounds are present in the environment by bio-methylation of inorganic lead and as a
results of the use for a long time of tetraalkylead compounds as antiknock additives in gasoline.

Robecke and Cammann [162] evaluated the HPLC behaviour of tetramethyllead (TTML) and tetraethyllead (TTEL) lead on a LiChrospher 60 column with different eluent mixtures. Both acetonitrile–LiClO$_4$ or methanol–LiClO$_4$ eluents showed that a 10% aqueous solution 0.1 M LiClO$_4$ is sufficient to achieve separation of TTML and TTEL by a ternary solvent (methanol–chloroform–LiClO$_4$) optimizes separation and reduces analyzing time (4 min). The method, applied to natural waters, gave, with normal pulse amperometric detection at a glassy carbon electrode, detection limits of 15.5 and 17 ng for TTML and TTEL, respectively. Shum et al. [140] achieved the separation of two alkylead (TTML, TTEL) and three organomercury species (methyl-, ethyl- and phenylmercury) using ion-pair chromatography and ICP-MS detection. The separation was made by a polyether ether ketone (PEEK) micro-column (5 cm×1.6 mm I.D.) packed with C$_{18}$ material and with an acetonitrile–water eluent containing ammonium pentanesulphate at 100 μl/min flow-rate. Detection limit was 0.2 pg and the method was validated with a freeze-dried urine reference material. The separation of TTML, TTEL and triphenyl-lead (TPhL) by ion-pair HPLC and ICP-MS detection was also obtained with a methanol–water eluent containing 4 mM sodium pentanesulphate [163]. By this approach the main problem encountered was the separation of TTEL from inorganic lead, in an attempt to optimise this separation a gradient elution was applied [164]. During the optimization of chromatographic parameters only inorganic and methyllead were strongly affected by a change of the ion pairing agent concentration and 8.0 mM was the optimum choice for resolution and for lowering the influence of salt concentration on the nebulizer tip and sampling orifice of the mass spectrometer. Inorganic, triethyl-(TEL), triphenyl-(TPhL) and tetraethyl-lead (TTEL) were well resolved with a gradient methanol concentration, from 40 to 90%, over 10 min. The high concentration of organic solvent gave a loss of sensitivity only for TTEL which was the last to elute and the relative detection limits were 2.8, 3.5, 77.5 and 7.4 ng/ml for TEL, TPhL, TTEL and inorganic lead, respectively. The method has been successfully applied to quality control sample, trace metals water supply of US Environmental Protection Agency (EPA) (Cincinnati, OH, USA). More recently Brown et al. [165] on the basis of the works of Al-Rashdan and coworkers [163,164] optimized the ion-pair HPLC separation of trimethyllead from Pb$^{2+}$, by a gradient program from 10:90 to 30:70 ratio of methanol to buffer (HAc/NaAc) between 4 to 7 min. Coupling the chromatographic system and ICP-MS by a single pass spray chamber with a concentric glass nebuliser a 0.48 ng Pb/g detection limit was obtained for trimethyllead ions.

On-column derivatization procedures have also been developed for simultaneous organic ionic lead and mercury species separation. Cammann et al. [166] developed an on-line enrichment on a RP-18 pre-column by adding the sample with methyl thioglycolate. Trimethyl-, triethyl- dimethyl- and diethyl-lead, methyl- and ethylmercury were separated on an Hypersil ODS column with a mixture of methanol and citric acid buffer. Detection limits in the range of 270 to 800 ng/l were obtained by spectrophotometric detection (UV 235 nm).

7.4. Mercury

A method has been developed based on the formation and separation of the methyl-, ethyl-, phenyl-, inorganic mercury complexes with ammonium tetramethylenedithiocarbamate [167]. An ODS RP-18 column was used and the elution was performed with acetonitrile–water–APDC buffered mixture. Detection was achieved by interfacing a glass flow cell between the chromatographic system and the cold vapour atomic absorption spectrometer. The on-column procedure in comparison with pre-complexation showed reduced detection sensitivity only for inorganic mercury which is very high in respect to the other species. The on-line procedure was chosen and after eluent optimization detection limits between 0.5 and 0.015 ng/ml were achieved, by coupling a sample (100 ml) preconcentration onto a C$_{18}$ microcolumn. N,N-disubstituted dithiocarbamate ligands: diethylidithiocarbamate (DDC), hexamethylenammonium (HMA)-hexamethylenedithiocarbamate (HMDC) and pyrrolidinedithiocarbamate (PDC) were tested for enrichment and separation of methyl-, ethyl-, methoxyethyl-, ethoxyethyl-, phenyl-
and inorganic Hg complexes [168]. A RP C\textsubscript{18} column was used and the best results were obtained with PDC complexes coupled with an acetonitrile–water buffered eluent. Analytes were determined by ultraviolet, post-column oxidation, cold vapour atomic absorption spectrometry (UV–PCO–CVAAS) [169]. Methyl- and ethoxyethylmercury coeluted but, since HCl pretreatment of samples decomposes the latter, it was possible to evaluate both species by analysing treated and untreated samples. Detection limits of 5.0 μg/l, obtained with the HPLC–UV–PCO–AAS system, were lowered to 0.5 ng/l with a preconcentration (300 ml samples) of mercury chelates on a microcolumn (Hypersil-ODS RP C\textsubscript{18}). Methyl-, ethyl- and inorganic mercury were well separated on a C\textsubscript{18} reversed-phase column with a methanol–acetonitrile–2-mercaptopropanoic acid eluent containing ammonium acetate [170]. The eluent composition was optimized in order to achieve the best chromatographic resolution and enabling ICP-MS detection through ultrasonic nebulization. Detection limit values (0.4–0.8 ppb) ten times lower than those obtained with LC–ICP-MS with a conventional nebulizer were obtained and comparable to those for LC–ICP-MS with cold vapour generation.

Munaf et al. [171] proposed a preconcentration and liquid chromatographic separation of methyl-, ethyl- and inorganic mercury based on microcolumns (Develosil–ODS and STR–ODS–H) with cysteine–acetic acid eluent. The cysteine concentration was the limiting factor because low concentrations are insufficient to elute mercury species and too large concentrations hinder the mercury–cysteine complex decomposition before the cold vapour detection. The sensitivity of the method was enhanced coupling a preoxidation step (with potassium peroxodisulphate and Cu catalyst) to the reaction for mercury vapour generation, in this way a 0.1 ng Hg detection limit was obtained. An ion chromatographic separation of methyl-, ethyl- and inorganic mercury as cysteine complexes was developed [172]. The eluent composition (acetic acid, sodium perchlorate and cysteine) was optimized with respect to the separation procedure and to the reductive reaction (NaBH\textsubscript{4}) which permits the detection of mercury with CVAAS. On-line preconcentration procedures were also investigated using both C\textsubscript{18} and ion-exchange microcolumn. The detection limits, for 100 ml samples, were 2, 10 and 4 ng for Hg, CH\textsubscript{3}Hg and C\textsubscript{2}H\textsubscript{5}Hg, respectively.

Tetra-n-alkylammonium bromide ion-pairing agents and sodium halides in methanol–water mixture were investigated as mobile phases for the separation of inorganic mercury and organomercury species (methyl-, ethyl-, benzyl-, phenyl-) [173]. The effect of TMA, TEA and TBA ions on the capacity factors of the species investigated was examined. The retention of Hg\textsuperscript{2+} was greatly dependent on the concentration of ion-pair reagent and its molecular size, and the capacity factor for Hg\textsuperscript{2+} increased with increasing both the parameters. In contrast to this behaviour organic mercury species showed lower capacity factor changes. The TBA ion-pairing agent was efficient for the separation of all the species and the addition of sodium chloride to the mobile phase gave better peak shapes and lower retention times. UV and DCP detection was compared, the sensitivities resulted in opposite behaviour and the detection limits ranged from 0.2 to 8.0 ng with UV and from 255 to 175 ng with DCP for benzylmercury and methylmercury species. Relatively high detection limits, for DCP, may be attributed to the high carbon content (organic solvent and ion-pair reagent) enhancing the background and low atomization efficiency due to larger ion-pair species difficult to penetrate into the DCP.

7.5. Tin

At the beginning of 1990 cyano phases (cyanopropyl-bonded silica gels) were used in normal-phase mode (eluents: hexane–acetonitrile–tetrahydrofuran) and tetraalkyltin compounds separated according to their polarity gave the following elution order: tetrabutyl-, tetraethyl-, tributyl-chloride, tetra-phenyl-, triethyl-chloride, diphenyl-dichloride and diethyl-dichloride [174]. The peak shapes and resolution were improved by an iodine chloride on-column pretreatment. UV detection (220 nm, time constant 50 ms) showed a complete separation in about 90 s with 6 ml/min mobile phase flow-rate. No details are given on detection limits and analytical dynamic range of detectable concentrations. Astruc et al. [175,176] made a theoretical and experimental study on on-line discontinuous detection in LC by graphite furnace atomic absorption
spectrometry (GFAAS) and its application to butyltin compounds at trace level. Separations of butyltin moieties have been obtained using a Nucleosil column with a 0.001% tropolone solution in toluene. The chromatographic procedure has been applied to tetrabutyl, tributyl-, dibutyl- and monobutyltin speciation in water. The first two analytes are coeluted and the last strongly retained onto the column. By coupling on-line detection by GFAAS the detection limit for dibutyltin was 10 ng/l. An highly fluorogenic reaction between triphenyltin (TPhT) and 3-hydroxyflavone in a micellar medium (Triton X-100) has been coupled with an ion-exchange chromatographic method (column: cation exchanger Partisil10 SCX, eluent: methanol−water, 0.15 M ammonium acetate) enabled to reach a detection limit of 0.02 ng for 200 μl sample injection [177]. The method was applied to the determination of TPhT in bottom seawater after enrichment on a C8 cartridge and gave satisfactory results at ng/l levels [178].

Cation-exchange chromatography of tributyltin and triphenyltin, usually performed with ammonium-acetate eluents, has been optimized by the addition of benzyltrimethylammonium chloride (BTMA) which also allows the indirect detection of trialkyltins [179]. The method has been applied to trimethyltin and triethyltin speciation in addition to the above mentioned compounds. The detection limits obtained (0.15–2.5 mg/l) were not so low, due to the kind of detector used, but the chromatographic approach is attractive.

Various types of couplings have been developed utilizing both inductively coupled plasma atomic emission spectrometry (ICP-AES) and direct current plasma atomic emission spectrometry (DCP-AES) for detection [180] or ICP-MS [181]. A reversed-phase LC (column: C8, 3 μm, 30×3 mm I.D.; eluent: methanol−water 5 mM sodium 1-pentanesulphonate) system has been optimized for trimethyl-, triethyl-, tripropyl-, tributyl- and triphenyltin separation and determination by ICP-MS equipped with an ultrasonic nebulizer [181]. Detection limits ranged between 2.8 and 16 pg Sn for various tin species and the entire procedure required less than 6 min.

Rivas et al. [182] investigated the effect of different spray chambers in HPLC–ICP-MS on the detection limits for organotin compounds. The instrumental interface, i.e., nebulizer and spray chambers, appeared the critical point.

7.6. Chromium

Discussions and reference lists for chromatographic techniques with off-line separation and preconcentration, or on-line methods for chromium can be found in Refs. [183–185]. The possibility of using a complexing agent in the mobile phase for the determination of chromium speciation has been shown [186–189] including reversed-phase chromatography after the formation of neutral chelates [190,191], IC [192] and ion-pair chromatography [193–198].

Studies on chromium speciation with ion chromatographic separation of its EDTA complexes involves, in addition to common parameters, a detailed evaluation of the temperature effect. This is due to the slow formation rate of the complex between Cr(III) and EDTA. An anion chromatographic separation (polymer-based anion-exchanger, eluent: EDTA−oxalic acid) has been optimized by working at a column temperature of 40°C [199]. Detection was made by direct introduction of eluate into an ICP-MS system. This method enabled 80–88 ng/l detection limits for Cr(III) and Cr(VI), respectively within a linear range from 0.5 to 5000 μg Cr/l and simultaneous determination of chromium speciation and Mn, Fe, Ni, Cu, Mg and Ca in water samples. Anion-exchange column containing a small portion of cation groups was also used for chromium speciation [200]. Examples of different approaches for chromium speciation with anion-exchange are two ion chromatographic procedures developed by Pobozy et al. [201]. In the first method (column: anion exchanger; eluent: potassium hydrogenphthalate) Cr(VI) was retained and Cr(III) was eluted in the void peak and oxidized post-column to Cr(VI). In this manner both Cr(III) and Cr(VI) were spectrophotometrically detected after post-column reaction with diphenylcarbazide (DPC). The second procedure was based on Cr(VI)–Cr(III) anion species separation after Cr(III) pre-complexation with 1,2-diaminecyclohexane-N,N,N′,N′-tetraacetic acid (DCTA). Detection limits evaluated for the first and the second method were 2.5 and 4.5 ng/ml for Cr(III) and 1.8 and 1.5 ng/l for Cr(VI), respectively.
Among ion-pair applications Posta et al. [196] optimised the Cr(VI)–Cr(III) separation on a RP C18 column by using a TBA–acetate, ammonium acetate, phosphoric acid and methanol based eluent at 2.5 ml/min flow-rate. The eluent composition enabled the chromium species separation enhancing the sensitivity of detection obtained by coupling HPLC to a flame AAS by a high-pressure capillary with an hydraulic high-pressure nebulization. Detection limits of 0.05 ng/l for both Cr(III) and Cr(VI) were reduced to 0.5 µg/l for Cr(VI) after a preconcentration step.

Since in all cases the main interest is in lowering detection limits and reducing analysis time, in chromium speciation great attention has been paid to the interfacing of ICP-MS detectors with HPLC separation modes. Jakubowski et al. [197] used ion-pair chromatography and hydraulic high-pressure nebulization with ICP-MS detection but, due to the presence of 25% methanol in the eluent, polyatomic interferences from carbon were a problem. Total chromium, Cr(III) and Cr(VI) speciation was achieved by Powel et al. [202] by coupling HPLC anion-chromatography (eluent nitric acid) with direct injection nebulization and ICP-MS. The detection limits obtained were 30 and 60 ng/l for Cr(III) and Cr(VI), respectively without particular chromatographic approach.

A procedure developed for Cr(VI) determination [203] has been modified by Byrdy et al. [204]. They used mixed-mode columns, namely IonPac AS7, for Cr(III) and Cr(VI) separation with (NH4)2SO4 eluent (pH 9.2). Cr(III) species was stabilized with EDTA before sample analysis and detection was performed both by ICP-AES and ICP-MS equipped with a high-performance interface and a concentric nebulizer. To avoid polyatomic interferences at m/z 52 from 36S16O7 due to the eluent, m/z 53 was chosen for detection. Relative detection limits were 0.40 ppb for Cr(III) and 1.0 ppb for Cr(VI) within a 4% R.S.D. and a linear dynamic range from 3 to 600 ppb and from 5 to 1000 ppb for Cr(III) and Cr(VI), respectively in aqueous media.

An automated on-line, two-column ion-exchange system has been proposed more recently [205]. Cation and anion species of chromium are sequentially retained by eluting samples through a chelating column (Chelex 100) and an anion-exchange (AG MP-1) column. Recoveries were obtained by eluting Cr(III) with 2.0 M nitric acid and Cr(VI) with a NH4OH–NH4NO3 mixture. Synthetic samples (75–350 ng/ml Cr) gave recoveries of 91% and 100% for Cr(III) and Cr(VI) respectively at a flame AAS detector, but anomalous results were obtained analyzing real samples e.g., tap water. It must be pointed out that this and similar methods more than chromatographic procedures could be defined as FIA ones.

8. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>atomic absorption spectroscopy</td>
</tr>
<tr>
<td>AES</td>
<td>atomic emission spectroscopy</td>
</tr>
<tr>
<td>BTMA</td>
<td>benzyltrimethylammonium</td>
</tr>
<tr>
<td>CP</td>
<td>cetylpyridinium</td>
</tr>
<tr>
<td>CTA</td>
<td>cetyltrimethylammonium</td>
</tr>
<tr>
<td>CVAAS</td>
<td>cold vapour atomic absorption spectrometry</td>
</tr>
<tr>
<td>DBF-Arsenazo</td>
<td>2-(2-arsenophenylazo)-1,8-dihydroxy-7-(2,6-dibromo-4-fluorophenylazo)naphthalene-3,6-disulphonic acid</td>
</tr>
<tr>
<td>DCP</td>
<td>direct current plasma</td>
</tr>
<tr>
<td>DDC</td>
<td>diethyldithiocarbamate</td>
</tr>
<tr>
<td>DDS</td>
<td>dodecyl sulphate</td>
</tr>
<tr>
<td>DEDTC</td>
<td>diethyldithiocarbamate</td>
</tr>
<tr>
<td>DIN</td>
<td>direct injection nebulizer</td>
</tr>
<tr>
<td>DTCA</td>
<td>1,2-diaminocyclohexanetetraacetic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>diethylenetriaminopentaacetic acid</td>
</tr>
<tr>
<td>ED</td>
<td>electrochemical detection</td>
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<tr>
<td>EGTA</td>
<td>ethylene glycol bis(2-aminoethyl ether) tetraacetic acid</td>
</tr>
<tr>
<td>GFAAS</td>
<td>graphite furnace atomic absorption spectrometry</td>
</tr>
<tr>
<td>HEDTA</td>
<td>hydroxyethylenediaminetetraacetate</td>
</tr>
<tr>
<td>HG</td>
<td>hydride generation</td>
</tr>
<tr>
<td>HIBA</td>
<td>α-hydroxyisobutyric acid</td>
</tr>
<tr>
<td>HMA–HMDC</td>
<td>hexamethyleneammonium–hexamethylenebishomocarbonate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HQ</td>
<td>8-quinolinol</td>
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<tr>
<td>IC</td>
<td>ion chromatography</td>
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<tr>
<td>ICP</td>
<td>inductively coupled plasma</td>
</tr>
<tr>
<td>IDA</td>
<td>iminodiacetic acid</td>
</tr>
<tr>
<td>IIC</td>
<td>ion interaction chromatography</td>
</tr>
</tbody>
</table>
IIR ion interaction reagent
IP ion-pair
IS ion spray
LA lactic acid
LC liquid chromatography
MBTAE 2-(6-methyl-2-benzothiazolylazo)-5-diethylaminophenol
MS mass spectrometry
ODS octadecyl silica
OS octanesulphonate
PADAP 2-pyridylazo-5-diethylaminophenol
PAPS pyridylazosulphoaminophenol
PAR 4-(2-pyridylazo)-resorcinol
PCO post-column oxidation
PCR post-column reactor
PDC pyrrolidinedithiocarbammate
PDCA pyridine-2,6-dicarboxylic acid
PDMA poly(butadiene±maleic acid)
PS-DVB polystyrene—divinylbenzene
REE rare earth elements
RP reversed-phase
TAN 1-(2-thiazolylazo)-2-naphthol
TAR 4-(2-thiazolylazo)resorcinol
TBA tetrabutylammonium
TDTMA tetraethyltrimethylammonium
TEA tetraethylammonium
THG thermochemical hydride generation
TMA tetramethylammonium
TPA tetrapropylammonium

References