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Review

Application of chromatographic and electrophoretic methodology to the speciation of organomercury compounds in food analysis

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Abstract

Trace metals such as mercury, especially its organic compounds, are an important risk to the environment and to man due to their accumulation in the food chain. For this reason, the routine determination of the very toxic methylmercury, and of other organic and inorganic mercury compounds in marine and land animals, vegetables, fruits and fresh water is of increasing importance in health and environmental control programmes throughout the world. The majority of speciation methods for organomercurials involve a series of fundamental steps for the identification and quantification of samples of biological origin: extraction or isolation from the matrix; derivatisation and concentration; detection; separation of different species of interest and of interference. Each of these steps, as part of the chromatographic analysis of MeHg and of other organomercurials is revised in this study using food samples.

Keywords: Reviews; Food analysis; Organomercury compounds

Contents

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1. Introduction; bioavailability; toxicity

Due to its natural geological abundance mercury (Hg) is widely spread in the environment as elemental mercury and as mercuric sulphide (cinnabar); industrial contamination stems from elemental Hg in lamps, batteries, thermometers and amalgams in the manufacture of chlorine, pesticides, fungicides, catalysts, paint pigments, etc. [1–3]. The increase in the emissions of Hg in the past years has led researchers to conclude that atmospheric Hg constitutes a man-made source of Hg and through methylation it may be converted into MeHg and accumulated in living organisms [4].

Hg exists in a variety of chemical forms that have different biological and environmental behaviours. The inter-conversion of these forms controls the environmental mobility of Hg and determines its biological properties. Two possible biogeochemical cycles of transport and distribution are possible [5,6]. A global one, involving the atmospheric circulation of elemental Hg vapour from its sources to the oceans, and a local one, which is dependent on the methylation of inorganic Hg resulting from industrial waste [7].

Mercury is also found in the environment as mercurious [Hg(I)] and mercuric [Hg(II)] cations. They appear in nature in the form of methylmercury (MeHg), dimethylmercury (Me_2Hg), and ethylmercury (EtHg), due to biological conversions in organic systems. The reverse transformation to inorganic Hg is more complex, but also occurs [8]. The methylation process is very important because of the highly toxic character of the methylated forms and it facilitates the inter-conversion between the different mercurial species; it generally takes place in sediments, oceanic and fresh waters [9–11]. Inorganic Hg and MeHg have also been found in aquatic organisms and in fish, constituting a great risk for human consumption [12–15]. Sulphate-reducing aquatic and bacterial micro-organisms are responsible for the methylation processes in cases which principally take place under anaerobic conditions [16], whilst the processes of demethylation appear clear in the presence of dissolved carbon [17–19]. The inter-conversion between the mercurial compounds and other species such as chloride concentration, sulphurs, humic and fulvic acids, are influenced by factors such as pH, redox potentials, organic matter and nutrient content, temperature, salinity and amount of oxygen [6–8,20].

It has been observed that in marine organisms the percentage of MeHg is much higher than in seawater (0.5–6%) [21]. At middle levels of the marine food chain levels of between 60 and 80% of MeHg with respect to total Hg have been found. In larger predators (tuna fish, tuna, swordfish, etc.) the amount of MeHg is 70–90% with respect to the total amount of Hg accumulated [21]. This is possibly due to its lipophilic character and the high fat content present in marine species [22].

If the process of bioaccumulation is associated with the man-made contamination, this could explain the massive intoxication produced since the middle of the century. In Japan with MeHg from an electric power station in Minamata in 1953–1960 and Niigata in 1964–1965 [5,23]. In Iraq in 1972 also with MeHg from fungicides containing EtHg [24,25]. Other pollution episodes at different sites in Western countries such as Sweden [26], Canada [27], USA [6] are almost always related with the accumulation of organic mercury in plankton and lake and bay fish [28].

Due to its lipophilic nature MeHg is accumulated in the animal and human food chain more easily than the inorganic forms of the Hg cycle. In general the major sources of human exposure to the mercurial compounds through diet are of marine origin (fish, shellfish and molluscs) [25]. Although, due to their use in agriculture and in the paint industry, their surrogate forms can appear in food products such as cereals, vegetables and fruit [29–32] and may be extended to animal products such as meat, milk or eggs, through the trophic chain [33].

The most common ways of intoxication by inorganic Hg are those resulting from an occupational exposure or the consumption of foods containing Hg. In a chronic intoxication apart from the non-specific symptomatology clinical symptoms such as mercurial stomatitis, mercurial erethism and trembling appear [34,35].

The toxicity produced by organomercury compounds varies according to their chemical form; they are more liposoluble than Hg(II) or Hg^0. This gives them better penetration in the cells, but they are also more rapidly metabolised [36]. On the other hand,

the alkyl compounds are more toxic than the aryl compounds.

In addition to the effects produced in the nervous tissue, the organomercurials and especially MeHg, can produce damage in non-nervous tissues, especially at an intestinal and renal level [37,38]. Studies carried out on humans and animals show that at the stage of central neural system development, this system is much more sensitive to damage produced by MeHg than in the adult stage [25,39], because this species is capable of inhibiting brain enzymes which are responsible for neuronal growth in the first stages of human development [38,40]. The phenyl derivatives have little toxicological activity and are irritants to the skin and mucous membranes. On the contrary, Me_2Hg has a high toxicity level, it easily penetrates the skin and may cause death [41].

2. Importance of the determination of organomercury compounds

The great importance of the biogeochemical cycle of Hg and of its mercurial forms (especially MeHg, because it is one of the most toxic) makes it necessary to continue to search and to develop methods of extraction and determination in matrices of widely diverse origin, such as biological tissues [42,43], human blood [44–46], urine [47,48], hair [49–51], natural gas [52–54], industrial oils [55], sediments [56–58], soils [59,60], air [61–63] aquatic plants [64,65], freshwater and saltwater [49,66–68], marine mammals [69] and above all fish [28,49,70–75]. For these reasons, certification studies in the past and present are being carried out to obtain concentration values of the samples. These will be certified and used afterwards as reference materials for the external evaluation of a laboratory within the quality guarantee programmes [76–80].

As a result of the intoxication episodes in Japan, the number of studies related to the speciation of organomercury compounds in food samples were increased because up to now the great majority of the quantitative methods used [81,82] were based on the determination of total mercury in those samples. Due to the high volatility and solubility in solvents shown by these compounds, chromatography is an adequate technique for their separation. It also allows combinations with different detection systems [83].

In order to obtain an efficient and exact determination it is important to select a sufficiently sensitive discriminating detection system. The key step is the pre-treatment of the sample to isolate interesting compounds from the matrix using a correct and efficient method. Over the years diverse procedures have been developed with this aim such as: liquid–liquid extraction by acid hydrolysis, liquid–liquid extraction by alkaline hydrolysis, distillation, solid-phase microextraction, extraction with supercritical fluids, extraction assisted by microwaves and derivatisation [84]. Table 1 shows different extraction and determination techniques that are used for the analysis of MeHg and other organomercurials in food samples from different origins. However, it should be mentioned that in some of these techniques such as gas chromatography with electron-capture detection (GC–ECD) a number of sources of error exist which lead to unsatisfactory results [85–87].

For these reasons it is necessary to continue to explore new methods that allow the improvement of this step in the separation and analysis of mercury compounds in biological matrices.

3. Application to samples of fish, shellfish and molluscs

3.1. Gas chromatography–electron-capture detection

GC–ECD has been the method of choice for the determination of MeHg and other organomercury compounds in marine-origin biological samples [33,88–91] for many years, together with indirect methods such as cold vapour atomic absorption spectrophotometry (CV-AAS) [59,73,92–94].

In GC, columns packed with different stationary phases were used [88,95–101]. However, all have shown some disadvantage, such as a low and/or variable response for MeHg, substantial peak tailing, as well as low selectivity against interference [102]. In the last decade different capillary columns have been evaluated with polar and non-polar stationary phases [92,101,103–105]. The columns with greatest phase thickness and low polarity are the most

Table 1
Different extraction and determination techniques for MeHg and organomercury compounds from food matrices

Species	Matrix	Sample preparation	Determination	Comments	Ref.
MeHg, Hg(II)	Tuna, dogfish	MAE (alkaline digestion)–hydride generation	HG–CT–GC–ETAAS	Comparison of three acids and two bases in MAE. Opened focused microwave	[145]
MeHg	Tuna (CRM463, CRM464), dogfish (DORM-1), mussel, clam, cockle	MAE (acid hydrolysis)	GC–ECD	12 simultaneous extractions. Closed-vessel microwave extraction	[137]
MeHg	Fish, mussel	Acid hydrolysis (iodoacetic acid)	HS-GC–MIP	Detection limit of 20 ng/g	[149]
MeHg	Water	BPh_4Na–CH_2Cl_2	GC–IT-MS	Detection limit of 0.1 μg Hg	[205]
MeHg	Dogfish (DORM-2, DOLT-2) Tuna (CRM463, CRM464)	Distillation (KCl–H_2SO_4) Alkaline digestion (TMAH)	GC–ICP-MS HPLC–ICP-MS	Study of artifactual formation	[86]
MeHg	Dogfish, tuna, mussel	Acid hydrolysis (HCl–cysteine acetate–toluene)	GC–ECD, GC–MIP-AES, CE	Comparative study. Different capillary columns used	[138]
MeHg	Fish (Dorm-1, CRM463, CRM464)	Acid hydrolysis (HCl–toluene–cysteine acetate)	CE (sample stacking)	Detection limit of 12 ng/g	[178]
MeHg	Dogfish, tuna	Distillation–H_2SO_4	HPLC–HPF-HHPN–ICP-MS	RP C_{18} pre-concentration. Detection limit of $1.25 \cdot 10^{-2}$ ng	[175]
MeHg	Water	$BNaEt_4$ derivatisation	PTI-GC–MIP-AES	Automatic and simultaneous speciation of metals	[201]
MeHg, Hg(II)	Dogfish, lobster	Alkaline digestion (KOH–MeOH)–$NaBEt_4$ ethylation	CT-GC–AAS	Detection limits of 4 pg (MeHg as Hg) and 75 pg (Hg^{2+} as Hg)	[143]
MeHg, EtHg	Swordfish, tuna	Acetone and toluene clean-up step–acid hydrolysis (HCl)–cysteine acetate–toluene	GC–DCP-AES	Comparative study with four solvents	[148]
MeHg, Hg(II)	Fish (DORM-2, TORT-2, DOLT-2)	Alkaline digestion (TMAH)–$NaBEt_4$ ethylation	GC–PAPES	Pre-concentration step in a Tenax-TA column	[156]
MeHg, EtHg, PhHg	Fish	Cu(II)–KI–toluene–clean-up step–benzene	GC–AAS	Detection limit of 0.1 ng/(as Hg)	[141]
MeHg	Dogfish, dolphin, lobster	Alkaline digestion (KOH–MeOH)–HCl–CH_2Cl_2–ethylation	GC–CV-AFS	Limits of detection ranged from 8–10 pg	[158]
MeHg and diorganomercury	Fish, water	Acid hydrolysis (HCl)–CH_2Cl_2	GC–APAN	Detection limit of 50 ng	[161]
MeHg	Swordfish, dogfish, lobster	Diatomaceous earth-HCl column–chloroform elution–sodium thiosulphate complexation	LC–AAS	Accuracy ranged from 94.4–99.6% Quantification limit of 0.6 μg/g (as Hg)	[164]
MeHg, Hg(II)	Water, fish	$BNaEt_4$–SPME	HS-GC–MS	Detection limit ranged from 7.5–6.7 ng MeHg/l	[16,197,198]
MeHg	Dogfish, tuna	Distillation–H_2SO_4	HPLC–UV–PCO–CV-AAS	RP C_{18} pre-concentration Detection limit of 0.04 ng/g	[174]
MeHg, Hg(II)	Water	Direct aqueous phase ethylation or Grignard reaction	CT-GC–QF-AAS	Detection limit of 0.1 ng/l. 10–20 min of analysis time	[205]
MeHg	Lobster, dogfish, tuna, cod, oyster, mussel, carp, trout, salmon, pilot whale, bull shark, whale blubber	Cu powder–H_2SO_4–KBr–toluene	GC–MIP-AES	Detection limit of 0.8 pg (as Hg)	[154]

Table 1 (*continued*)

Species	Matrix	Sample preparation	Determination	Comments	Ref.
MeHg, EtHg, PhHg, Hg(II)	Mussel, tuna	(1) Direct aqueous phase ethylation (2) Chelation–extraction+ Grignard reaction	GC–MIP-AES	Comparison of different methods of derivatisation	[152]
MeHg	Haddock, pike, cod, perch, eggs, chicken, liver of pig	Acid hydrolysis (HCl–benzene–cysteine, 2-mercaptoethanol or aqueous solution of sodium sulphide	GC–ECD	Addition of an excess of mercuric ions	[112]
MeHg, EtHg, PhHg	Fish, shellfish, rice, strawberry, peppers	Acid hydrolysis (HCl)–benzene–aqueous glutathione solution	GC–ECD	Study of 16 kinds of column packings	[107,182]
MeHg	Dogfish, salmon, trout, tuna, seal, beluga, crab, herring, etc.	$CuSO_4$–NaBr–toluene–sodium thiosulphate complexation–KI extraction	GC–ECD	Recoveries around 99% iodine decomposition	[95,118]
MeHg	Tuna, cod, mackerel, oyster, clam, lobster, shrimp	Acetone clean-up step–acid hydrolysis (HCl)–benzene	GC–ECD	Does not require a cysteine clean-up step	[120]
MeHg	Swordfish, dogfish, shrimp, clam, oyster, canned tuna	Acetone and toluene clean-up step–acid hydrolysis (HCl)–toluene	GC–ECD	Method Official. First action of AOAC. Isopropanol to reduce emulsions	[99]
MeHg	Tuna (CRM463, CRM464), cockle dogfish (DORM-1), mussel, clam	SFE (acid hydrolysis)	GC–ECD	SFE reduced sample manipulation	[91]

adequate. However, the need for conditioning of most of these columns with a solution of mercury(II) chloride [97,104,106–110]; potassic iodide [95]; mercury(II) iodide [96] or methoxyethylmercuric iodide [108] in order to obtain satisfactory separations with reproducible results, is sufficiently evidenced.

Following the method of preparation of a sample proposed by Gage, who used acid hydrolysis of the biological material with HCl prior to extraction of MeHg and PhHg with benzene [111], two independent studies were initiated. Their aim was the separation and determination of organomercury compounds using GC–ECD.

Westöö applied his method to the determination of MeHg in fish and other foods [106,112,113]. He tested two different methods of performing the extraction of MeHg in aqueous phase:

(1) By initial addition of an excess of mercuric ion (5% $HgCl_2$) to eliminate possible interference caused by the thiol groups.

(2) Synthesis of a water-soluble sulphur compound of methylmercury. It was performed with the initial extract of benzene using sodium sulphur, 2-mercaptoethanol or cysteine in the absence of chromatographic interference, the latter one giving the best results. The chromatographic column used was packed with a Carbowax 20M type phase.

Sumino separated the organomercury compounds in fish and seafood samples using hydrolysis with HCl–extraction with benzene–re-extraction with an aqueous solution of glutathione; he evaluated 16 different stationary phases in a detailed study of the use of GC–ECD and only obtained good results with highly polar phases [107]. The method was applied to foods related to the marine-origin intoxication in Minamata, and performing experiments on animals, to which he administered MeHg.

The procedure for preparing samples used by Westöö with some modifications was the one recommended by the US Environmental Protection Agency, even though it is a quite lengthy procedure. The selection of solvents was limited by the use of ECD and losses were due to the large number of extractions.

Thus, in order to prevent the formation of emulsions during the extraction of MeHg in samples of fish with high fat content, Newsome [114] proposed a modification using an extraction with KBr in HBr–benzene. Afterwards he performed a re-extraction with aqueous cysteine which, after acidification with HBr, was again extracted with benzene. This extract

was analysed using GC–ECD obtaining a recovery of 95% from fish tissues.

The method proposed by Westöö was the most frequently used. It was used in fish samples, which were analysed using GC–ECD with a packed Carbowax 20M column [115]. It was applied in several samples with a detection limit of 0.01 ppm of MeHg from molluscs [116] and 0.08 ppm for MeHgCl from tuna and swordfish [108]. The chromatographic separation (GC–ECD) of different alkyl- and arylmercury types with columns of phenyldiethanolamine succinate at 5% was also studied [108]. The latter method was later used in the analysis of MeHg in samples of mussels and new species of fish in the Adriatic Sea [117].

In their endeavour to improve the extraction of mercury compounds in food type biological samples, Uthe et al. [95] proposed a new method based on the release of MeHg with $CuSO_4$ and NaBr, extracting the BrMeHg with toluene (less toxic than benzene). Prior to chelating with thiosulphate, it was extracted with a potassium iodide (KI) hydro–alcohol solution and finally determined using GC–ECD. By this method applied to fish (salmon, shark, pike, trout, tuna, herring, seal, crab, etc.) [95,118] they managed to reduce analysis time and the number of steps and obtained better recovery levels (99%) than with the method used by Westöö. However, it showed problems such as the formation of emulsions which in some samples were persistent and a marked facility for the decomposition of MeHg iodide by light.

The need to clean the chromatography column with injections of KI was also observed, as contamination appeared in the ECD system. Even then, this procedure was followed for the determination of MeHg in fish (eel, etc.) [70,96]; it was necessary to clean the samples with a compound with free –CH groups such as cysteine [96]. Years later, arctic fish and mammal tissues were treated analogously in the analysis of MeHg using GC–ECD [69]. In these cases, the amount of MeHg found exceeded the levels permitted by the Canadian Federal Consumption Guideline for Hg in fish. This together with important differences in toxicity for inorganics and organics, and the fact that some of these mercury compounds may be transferred from the mother to the foetus, reveals the importance of performing Hg speciation in tissues that are used as food source.

Ealy et al. [119] studied the separation, identification and determination of MeHg, EtHg and methoxyEtHg(II) in the form of halides in fish samples. The procedure consisted in keeping the sample for 24 h in KI solution after an equilibration of the aqueous phase during 2 min with an equal amount of benzene and the subsequent injection in the GC–ECD system of an aliquot of benzene extract. The stationary phase of the column was 5% cyclohexane–dimethanol–succinate. The extraction steps were monitored with RHgX halides labelled with ^{203}Hg.

In 1976 a method was published in which the cleaning step using cysteine, as proposed in the method of Westöö [120], was eliminated. With a simple washing of the sample (tuna, cod, mackerel, oysters, clams, lobster, prawn) with acetone, other organic molecules which could interfere in the chromatographic method (GC–ECD) were eliminated. The samples, acidified with HCl, were extracted with benzene. The detection limit observed was 0.10 ppm. This process was sensible to the production of chromatographic interference by organic species which had not been eliminated in the washing step with acetone and which afterwards could be co-extracted in benzene together with MeHgCl.

Some years later this extraction technique was satisfactorily applied to fish samples and shellfish (tuna, shark, sailfish and shrimp) [121]. The determination of MeHg was performed using GC–ECD with a column of 5% diethylene glycol succinate (DEGS) previously conditioned with mercury(II) chloride. A detection limit of 0.0016 μg/ml was obtained.

However, the demand for a fast, sensitive and adequate method of analysis for the isolation and routine analysis of MeHg in biological samples continued to exist. Cappon and Smith [88] extracted MeHgCl, EtHgCl and PhHgCl in the form of bromide from different samples, among which were fish, which they routinely determined at the rate of 24 samples per day using GC–ECD. A recovery rate of between 70 and 90% was obtained, with a detection limit of 1 ppb. It was also later applied [90] to fish and mussel samples carrying out the extraction of MeHg in a solution of sodium thiosulphate with benzene, after addition of KBr. A decade later, Filippelli [33] proposed an extraction method which

he compared to that of Cappon and Smith [88]. It consisted of speciation using GC–ECD of MeHg, EtHg and PhHg in different samples of fish (tuna, sardine, anchovy, oysters) chelating these compounds with an aqueous solution of thiosulphate and $CuCl_2$ prior to extraction with benzene.

Finally, in 1983, Hight and Capar [110] developed a practical technique for the separation and determination of MeHg in fish and shellfish (swordfish, tuna, oysters, prawns) applicable to routine analysis. The cleaning process recommended by Watts et al. [120] was used, substituting the filtration process for an agitation one, centrifuging and decanting. This saved time and eliminated possible losses. They used a chromatographic column packed with a stationary phase of 15% DEGS conditioned according to the description of O'Reilly [97] with $HgCl_2$. The detection limit obtained with the GC–ECD system was 0.05 μg/g as Hg.

Another system of preparation of tuna and swordfish samples was finished at the same time [122]. The homogenised fish tissue was treated with KBr to release the MeHg and the MeHgBr was extracted with methylene chloride. A cleaning step was performed with cysteine acetate and the MeHg was re-extracted with toluene for future determination by GC–ECD. A column packed with a stationary phase of 10% DEGS–1% $H_3PO_4$was used. This method was proposed for routine analysis, although the average time for determination was 1 h. The use of KBr was recommended instead of HCl, because the latter produces emulsion problems in the extraction step with methylene chloride. This author also conditioned the chromatographic column with a solution of $HgCl_2$ in acetone before performing the analysis. Sensitivity was kept constant for 2 or 3 days. The recovery level obtained was 95.6%.

The same type of acid extraction HCl–KBr–$CuSO_4$–aqueous cysteine–toluene was proposed. A study was made on the decomposition of MeHg in hydrochloric medium when organic solvents were used [123]. The use of KBr and $CuSO_4$ instead of the more usual NaCl slightly reduces decomposition. However, light is the major factor responsible for many MeHg decomposition processes. For this reason it was convenient to protect the samples and extracts from light. This method, together with analysis using GC–ECD, was applied to fish samples and the results obtained for MeHg were compared with those of total Hg provided by voltametric analysis.

In 1984, Alvarez et al. [89] made an evaluation of the method proposed by Hight and Capar [110] for analysis by GC–ECD of frozen products such as shellfish, fish and pre-packed fish. They obtained levels of recovery in the range of 95.7–114% after performing additions of standard aqueous solution of MeHgCl. The method was based on successive washings of the samples (prawns, clams, scallops, prepared crab, cod fingers and filets, whiting fillets, etc.) with acetone, acidification with HCl and extraction with benzene. The cleaning step with cysteine was not required. A chromatographic column packed with a stationary phase of 5% DEGS conditioned with $HgCl_2$ was used.

Hight and Corcoran published a developed method [99] and a collaborative study [124] for the rapid determination of MeHg in fish and shellfish (swordfish, shark, prawns, clams, oysters and tinned tuna) by GC–ECD. A year later a first Official Action of the method was published [125]. This consisted in performing several washings of the sample with acetone and toluene to eliminate organic interference, cleaving of MeHg bonded to the proteins using HCl and subsequent extraction with toluene. To reduce the possible formation of emulsions 1 ml of isopropanol was added. The toluene extract was analysed by GC–ECD with columns of 5% DEGS and a conditioning of $HgCl_2$ solution al 1% in toluene. Recovery rates of 100.5% were obtained with a quantification limit of 0.25 μg/g in the first study [99] and between 86 and 98% in the collaborative study with eight laboratories [124].

Another modification of the method proposed by Westöö was carried out by Horvat et al. [92]. They obtained excellent results in the process of eliminating organic interference, thus preventing the formation of emulsions. For this the MeHgBr was formed in the samples (fish and molluscs among others) upon treatment with HBr and was extracted with toluene. Afterwards it was selectively absorbed in paper impregnated with cysteine acetate that was subsequently washed with toluene. GC–ECD with a column of 5% DEGS–PS was used as a detection system, achieving a detection limit of 0.1 ng/g.

Based on the method of Westöö [106] and of

Hight [124] four speciation studies of organomercury compounds were performed [126]: separation by GC–ECD, in accordance with these two techniques, for the preparation of MeHg samples in molluscs; a control of the quality of the method of Hight combined with the GC–ECD system for MeHg; a search for an alternative methodology for GC; environmental studies in samples of molluscs from different parts of the Galician coast.

An exercise of inter-calibration between 13 laboratories on mussel and shark samples supplied by the National Research Council of Canada [127] was carried out using different detection systems. Various methods were used for the treatment of the samples, such as acid hydrolysis as used by Westöö [106,112], extraction with toluene following treatment with $CuSO_4$ and NaBr and re-extraction with sodium thiosulphate [95] or with cysteine and toluene [128], among others. Thus, certification of reference materials has been carried out, which will be used extensively once the methods have been validated [76]: DORM-1 and DORM-2 (shark muscle), DOLT-1 (shark liver) and TORT-1 and TORT-2 (lobster hepatic-pancreas).

In 1987, the Standards Measurement and Testing Programme of the European Commission (initially BCR) set up a project in which the first step was a few comparative exercises. As a result of which two new certified reference materials of tuna for MeHg: CRM-463 and CRM-464 [80] appeared. Other organisations have also contributed to the production of certified reference materials in biological tissues. One of these is the International Atomic Energy Agency (IAEA) which has prepared a mussel (IAEA-350) with a high MeHg content and another mussel (IAEA-142) with a low MeHg content [129]. Another is the National Institute of Standards and Technology (NIST) which has certified MeHg in mussel materials (SRM-1974a, SRM-2974 and SRM-2976) [130]. In all these cases the determinations were performed by different methods of analysis, among which was GC–ECD.

The problems shown by the chromatographic columns during the analysis of biological samples (mussels, cockles, clams, scallops or tuna) made it necessary to improve the determination methods for organomercurials. These improvements were made in different ways. Either in the technique of preparing the sample using butylation with a Grignard agent [103], or by using capillary columns in GC–ECD [101,103,104]. In some cases, and due to the exceptional thickness of the stationary phase of the columns, either they did not require conditioning [131] or treatment with HBr was used, which neither damaged the columns (packed and capillary) nor the ECD system [132].

On the other hand, other techniques for the extraction of MeHg in fish and mollusc samples have been attempted. This is the case of alkaline digestion associated with determination using GC–ECD. One of the first applications of this process was performed on mussels from the Pontevedra and Arosa Estuaries (Galicia, Spain) [133]. Alkaline hydrolysis for the extraction of MeHg used KOH. After complete digestion, HCl was added and MeHgCl formed was extracted with benzene. This compound was chelated prior to the use of cysteine as a purification step and was re-extracted with benzene. The analysis was carried out using GC–ECD. Although flame ionisation detection (FID) was tried, it showed no response, which was to be expected for a substance with the chemical nature of ClMeHg. Cappon and Smith [134] also made use of this technique of sample preparation. This was treated with an aqueous dissolution of sodium hydroxide at 45%, cysteine hydrochloride at 1% and NaCl at 1%. Afterwards, the analysis was effected by GC–ECD.

A solution of cysteine was used as a chelating agent to maintain the stability of the carbon–Hg link and to avoid losses of MeHg at high pH values due to the formation of metallic hydroxide [135].

In another recent study the correlation between 32 fish samples for human use containing MeHg and the accumulation of MeHg in human hair from 20 individuals was tested [136]. The fish samples were treated with an alcoholic solution of KOH followed by extraction with dithizone-benzene and analysis using GC–ECD.

The process of alkaline digestion causes the breakdown and release of MeHg in quantitative form, with high recovery levels in solid samples. However, only a small aliquot of the solution may be subjected to derivatisation with $NaBEt_4$ due to the significant interference produced in the matrix. Therefore, large concentrations of MeHg were required in the sample [85,129]. A second problem

arises if the concentration of inorganic Hg is high, because under these conditions the derivatisation reactant could transform the inorganic Hg in MeHg, originating a systematic error in the determination [78,86,87].

New alternative techniques of MeHg sample preparation in fish and mollusc matrixes (tuna, shark, mussel, cockles and clam) have been developed in order to reduce extraction time, to simplify the process, and to reduce waste of solvents and samples. Two of these techniques are supercritical fluid extraction (SFE) [91] and microwave-assisted extraction (MAE) [137]. The advantages and disadvantages of both techniques, with respect to manual acid hydrolysis, has been commented [138]. SFE and especially MAE showed recovery levels comparable to conventional acid extraction, but were more precise. This together with the previously mentioned practical benefits, gives the MAE and SFE procedures a clear advantage when compared to manual extraction. Using SFE, the extractions are made in less than 45 min with a few ml of solvent. Also, MAE allows the simultaneous extraction of up to 12 samples in a maximum of 10 min. This makes it a very interesting selection for routine analyses of MeHg in biological samples of marine origin.

3.2. Gas chromatography–atomic absorption spectrometry

One of the first studies [139] was based on the separation by GC of organomercurials (MeHg, EtHg) in fish, by either oven or flame combustion of the column effluent and the subsequent measurement of the resulting atomic vapour by atomic absorption spectrometry (AAS). The procedure of Longbottom et al. [96], with some modifications which favour sensibility, was used. The process of acid hydrolysis (HCl), which does not require the cleaning step, was used for the preparation of the sample and the extraction was performed with benzene.

The GC–AAS technique was also used in the determination of mercury compounds in fish, but with a different sample preparation method. This consisted in the addition of Cu(II), acidification with KI and subsequent extraction with toluene. After this, a cleaning step and final re-extraction in benzene of the corresponding iodide followed [140]. A decade later the speciation of MeHg, EtHg and PhHg by GC–AAS in samples of fish according to the process proposed by Bye was performed with a single benzene extraction. The detection limit was 0.1 ng/g Hg and recovery levels of 95% were obtained [141].

The derivatisation of the analytes by the generation of hydrides with $NaBEt_4$ was also used for the quick, precise and exact determination of MeHg in fish samples (shark and lobster) after applying acid hydrolysis [142] or treatment with a methanolic solution of KOH [143]. The determination was performed with a cryogenic trap in the second case.

The extraction of Westöö for MeHg was also applied in samples of codfish, prior to determination using the coupling of two systems: GC–ECD–CV-AAS [144].

In an inter-laboratory study carried out in 1993, diverse methods of sample preparation were used, including ethylation. Important differences appeared in the MeHg concentrations measured in mussels and tuna. This confirmed the need for a new independent method such as that mentioned in the study (ethylation followed by GC–AAS) [77]. This method was an excellent candidate for the determination of species [Hg(II), MeHg, Me_2Hg] in environmental samples, as the results in relation to other ethylation studies showed.

A fast, simple and exact method has recently been developed for the preparation of samples and the simultaneous determination of inorganic Hg and MeHg in tuna and shark tissue [145]. MAE was used in combination with an automatic inter-phase of hydride generation–cryogenic trap–gas chromatography–detection by electrothermic atomic absorption spectroscopy (HG–CT–GC–ET-AAS). All these techniques, when combined, produce quick and reliable results due to the significant reduction in the number of analytical steps and potential sources of error. The efficiency of the extraction of three acids (HNO_3, HCl and CH_3COOH) and of two bases [tetramethylammonium hydroxide (TMAH) and KOH–MeOH] has been compared in this study. The results obtained confirm that the microwaves extraction with either of the two bases, simultaneously produces optimum recovery levels for Hg(II) and MeHg.

3.3. Gas chromatography–atomic emission spectrometry

Different authors have applied GC coupled to atomic emission spectrometry (AES) [146], using plasma induced by microwaves, as a detection system (GC–MIP-AES) for the determination of organomercury compounds. Detection using atomic emission is advantageous in the case of methyl elements, because its intense emission and low spectral background provides excellent sensitivity and a high degree of selectivity [146]. Also the chromatographic problems showed by the separation of mercurial halides, the passivity of the capillary columns, etc., led to search for a satisfactory alternative. This is the way, salts of MeHg in fish tissue samples were separated [147].

Another combination that has been performed is that of GC with direct current plasma and atomic emission spectrometry (DCP-AES). The result was a simple, rapid and cheap system for the determination of MeHg in fish (swordfish, tuna) [148]. The method proposed by Hight and Corcoran [99] was used for preparing the sample, four different solvents and a column packed with 5% DEGS at 115°C, allowing the separation of MeHg from EtHg in less than 5 min. The results were compared with those provided by the GC–ECD system and some disagreements were found. Toluene did not appear to produce peaks of interference in GC–ECD. Acetone and diethyl ether produced the formation of artefacts, which were proportional to the increase in injection temperature. These and other problems in the GC–ECD system might show are avoided by using the GC–DCP-AES system, which is more precise for the determination of MeHg in complex matrixes.

The determination of MeHg in biological samples using headspace (HS) for the injection of the sample into the chromatographic column was improved [149]. The MeHg iodide obtained after acidifying the tissue (tuna and mussel) with sulphuric acid and treating it with iodoacetic acid, was detected with a MIP-AES system. The preparation of the sample was accomplished in a closed vial, the same one used in headspace, and the detection limit was 20 ng/g of tuna.

Derivatisation with Grignard agents has also been used, prior to determination using GC–MIP-AES. This avoids problems of passivity in the column and improves the chromatographic characteristics of the species to be separated, such as MeHg and EtHg [103] or Hg(II) and MeHg [150] in fish samples.

The process of acid hydrolysis proposed by Hight and Corcoran [99] with certain modifications [131] was selected for the application of a direct determination method for MeHg using GC–MIP-AES in samples from different origins among which shark, tuna, mussel, cockles and clam [151] stand out. Two capillary columns at a programmed temperature were tried. One of dimethylsiloxane (HP-1) and the other of 5% diphenyl–95% dimethylsiloxane (HP-5). The latter had greater phase thickness, which made it more resolution efficient, and with longer duration of the conditioning with 1% $HgCl_2$. Detection limits of 1.5 pg (HP-1) and 1.2 pg (HP-5) and quantification levels of 4.5 pg (HP-1) and of 2.6 pg (HP-5) were obtained. Also, the use of this detection system guaranteed the correct separation (less than 4 min) and quantification of MeHg for unclean samples or samples which had a high fat content (mussels) presenting serious chromatographic resolution problems under the GC–ECD system.

On the other hand, the efficiency of different derivatisation processes (direct ethylation in aqueous-phase, direct phenylation in aqueous-phase, butylation using Grignard reagent), have been compared using GC–MIP-AES [152] in the multi-element determination process of various organomethylates. Among which were MeHg, EtHg, PhHg and Hg(II) in samples of biological tissues such as mussel and tuna

A method for the determination of MeHg present in nine fish species has been described [153]. It used aqueous-phase ethylation with sodium tetraethylborate (STEB) in a buffered KOH–MeOH digested fish sample. The derivatised MeEtHg was purged from solution into a graphite carbon trap. It was then thermally desorbed and collected in a cryogenic trap prior to analysis by GC–AES. The method provides for increased sample turnover by the use of permeated Me_2Hg for MeEtHg response calibration.

Following the extraction procedure of Jiang et al. [141] with modifications, a method of MeHg quantification was developed using GC–MIP-AES [154], validated and applied to marine biological materials. Certified reference materials were used and a solid–

liquid extraction procedure, under acid conditions, was proposed for the selective extraction of various alkylmercury species. The conditioning of the chromatographic column and the derivatisation of the analytes was not necessary, if columns with high phase-width were used.

Likewise, an investigation on the interference of sulphur in the quantification of MeHg in marine tissues (TORT-1, DOLT-2, IAEA-350 and DORM-2) was carried out using GC–MIP-AES [155]. The addition of Cu(II) in powder to the samples before acid extraction with H_2SO_4–KBr–toluene allowed separation of the sulphate species in the samples, avoiding interactions between these and the organomercury analytes.

An alternative to the MIP-AES system coupled to GC was a helium plasma produced at atmospheric pressure in a conventional graphite oven used for AAS (PAPES). This source can be configured with a graphite or metal electrode and with an external graphite or ceramic tube, operating at 50 to 200 W, from room to high temperatures. For these reasons the GC–PAPES system shows a much longer lifecycle than GC–MIP-AES. The GC–PAPES system has been applied to reference materials of fish tissue (DORM-2, TORT-2 and DOLT-2) [156]. The samples were digested with TMAH and the ionic species purged from aqueous solution after ethylation with $NaBEt_4$. Then they were pre-concentrated in a column with Tenax-TA and thermally desorbed at 90°C in a chromatographic column buffered with 15% OV-3 in Chromosorb W. The detection limit found for MeHg was 7 pg.

Similarly, the analytical potential of the radio frequencies of the incandescent discharges has been investigated for the speciation of low levels of MeHg, EtHg and Hg(II), in fish tissue samples. AES was used as the detector coupled to GC after extraction with sodium diethyldithiocarbamate and derivatisation by Grignard [157].

3.4. Gas chromatography–atomic fluorescence spectrometry, gas chromatography–mass spectrometry and others

GC coupled to atomic fluorescence spectrometry (GC–AFS) is a technique that has been used for the determination of organomercury compounds in environmental samples since the 1980s. However, it is less used in food samples. Very recent references can be found among the studies carried out in this field. Thus, Liang et al. [158] developed a method for the determination of MeHg in fish samples using GC–CV-AFS. An alkaline digestion was performed with 25% KOH in MeOH at 75°C followed by acidification with HCl prior to extraction with methylene chloride. The MeHg was re-extracted in aqueous phase for ethylation. The main advantage of the method was the easy isolation of the MeHg from the matrix without the need of cleaning steps. However, to avoid interference during ethylation it was necessary to work with very small amounts of sample. The recovery levels obtained were close to 100%.

The isothermal GC–AFS may be found among the methods selected by the different laboratories participating in numerous studies, for the certification of marine reference materials (DORM-1, DORM-2, TORT-1) [129]. In all cases, alkaline digestion with methanolic solution of KOH, followed by ethylation in aqueous phase and in-column-trapping, which in some studies was performed at room temperature [159], was the procedure used. In other cases, the mussel material to be certified (SRM1974a, SRM2974 and SRM2976) was buffered in closed PTFE vials during the night at 70°C with 25% KOH in MeOH. After the MeHg was derivatised with $NaBEt_4$, separation and detection was carried out using isothermal GC followed by pyrolysis and CV-AAS [130].

The GC–AFS system was also used in the certification of tuna CRM463 and CRM464, associated with HS. The extraction of the MeHg of the samples was done with H_2SO_4–CH_3COOH [80].

Another technique which is being introduced in the process of fish sample preparation for analysing MeHg, due to its simplicity and speed, is solid-phase microextraction (SPME). It is based on the extraction of volatile or semivolatile organic compounds, directly from the aqueous or gaseous sample, through a capillary or fused-silica fibre that is held together with an appropriate stationary phase. In this case an aqueous fish extract in KOH has been used, $NaBEt_4$ added to derivatise, and the fibre introduced with dimethylsiloxane as the stationary phase. The processes of ethylation and extraction with the fibre were simultaneous, so the time saved

is considerable. Immediately afterwards the mercury derivate was introduced in a GC–AFS system using a splitless injector [160].

On the other hand, mass spectrometry has been coupled to GC (GC–MS) for the determination of MeHg in samples from different origins. It has been performed by Cai et al. [16] in fish samples, after being derivatised with $NaBEt_4$ and SPME.

In a study for detecting the formation of artefacts in environmental samples as a function of the extraction process used, alkaline digestion with TMAH and distillation using distilled water vapour and addition of KCl and H_2SO_4 were used in the determination of MeHg, in samples of fish (reference materials DORM-2, CRM463 and CRM464), prior to detection with a GC–ICP-MS [86] system.

Another detection system used in the analysis of MeHg in fish and mussels was microwave-induced plasma detection, HS-GC–MIP [149]. With this method time was saved and the degradation of the chromatographic column, which occurs with direct injection, was avoided. The extraction process used was based on the one described by Decadt el al. [43] in which the sample was treated with iodoacetic acid and the iodide form of MeHg was detected afterwards.

The inter-phase between the GC and the atmospheric pressure active nitrogen detection (GC–APAN) systems has been used for the separation and detection of MeHg in fish samples [161]. It is a sensitive and selective detector for Hg species monitored in the atomic line of 253.7 nm. The samples were treated with HCl and the MeHg extracted with methylene chloride. The emulsions formed between aqueous and organic phases were eliminated by vacuum filtration.

3.5. (High-performance) liquid chromatography

Liquid chromatography (LC), as a separation technique coupled to a selective detection system is without doubt an alternative for the determination of organomercury compounds because it simplifies sample preparation. The most used detection systems are electrochemical detection (ED) and CV-AAS. The development of high-performance liquid chromatography (HPLC) combined with different detectors has been an advancement in this field.

One of the first investigations made on samples of tuna and swordfish was carried out at the end of the seventies [162] using an electrochemical liquid chromatography detector with an amperemetric electrode amalgamating Hg with Au for the analysis of MeHg, EtHg and PhHg. A reversed-phase column of C_{18} was used and the mobile phase was water–MeOH (60:40) with 0.06 mol/l of ammonium acetate (pH 5.5) and $5 \cdot 10^{-4}$ mol/l of 2-mercaptoetanol as chelating agent. Alkaline digestion was used for preparation of the sample and after acidifying with HCl the organomercury compounds were extracted with toluene as a neutral chlorate chelate. A re-extraction in aqueous phase was then performed with solution of sodium thiosulphate and ammonium acetate. The detection limit for MeHg was 40 pg.

To avoid sample preparation problems (losses or emulsion formation, excessive manipulations) and to increase resolution a method based on treatment with HCl, followed by chloroform elution in a column packed with diatomaceous earth was used. An aqueous phase re-extraction was performed, chelating the MeHg with sodium thiosulphate. This solution was then separated using HPLC with ED or AAS [163]. A Zorbax ODS column was used with MeOH–ammonium acetate (3:2) with mercaptoethanol to pH 5.5 as mobile phase. The method was applied to different fish and molluscs samples (sardine, tuna, swordfish, whiting, turbot, prawns, squid and octopus). This method (HPLC–AAS) was then used in a joint study on samples of tuna, lobster and swordfish and adopted in a preliminary Official Action [164]. Some years later it was used in a study carried out by the US Food and Drug Administration (FDA) in 220 samples of tinned tuna fish collected in 1991 [22]. The samples were selected to represent different criteria (colour, class, compaction grade, etc.). The average amount of MeHg found was 0.17 ppm (expressed as Hg) ranging between 0.10 and 0.75 ppm and none of the samples exceeded the FDA action limit of 1 ppm.

Similarly, a HPLC method inter-phased with CV-ASS was developed and evaluated for MeHg, EtHg and Hg(II) using dolphin liver samples [165]. These compounds were separated as complexes with cysteine, after treatment with NaCl–HCl and separation with a reversed-phase column and mobile phase of 40 mmol/l of cysteine in 0.1 mol/l of acetic acid (pH 2.9).

A modified version of the method proposed by

Westöö was also used in fish sample preparation for the separation by HPLC–CV-AAS of MeHg, EtHg and PhHg [166].

In another study, using HPLC–CV-ASS on tuna fish samples, speciation of MeHg, EtHg, PhHg and Hg(II) [167] was performed. The samples were treated with a solution of thiourea and after centrifuging and filtering, complexes were formed with ammonium pyrrolidine dithiocarbamate (APDC). Pre-concentration was performed in reversed-phase column C_{18}, used later for separation, and CH_3CN–water (58:42) containing $5 \cdot 10^{-4}$ *M* APDC as mobile phase.

The mass spectrometry of plasma coupled to induction (ICP-MS) is a trace metal detector which offers better sensitivity and multi-element detection capacity as compared to AAS or ICP-AES [168]. For this reason it was selected as the detection system after separating with LC different organomercury compounds in a tuna fish sample (NBS RM-50) [169]. The samples were washed with acetone to separate fat, treated with 1% NaCl in HCl, and the corresponding mercury compound was extracted with toluene, re-extracting afterwards with mobile phase (0.06 *M* ammonium acetate–0.005% 2-mercaptoethanol–3% acetonitrile).

HPLC–ICP-MS has been one of the techniques selected for studying the formation of MeHg from Hg(II) added to fish samples (DORM-2, CRM463 and CRM464) using various extraction processes such as distillation or alkaline extraction [86].

Using HPLC, with UV spectroscopy at 254 nm as the detection system, analysis of MeHg has been performed in samples of molluscs (cockles, mussel) obtained from different coastal areas of Galicia [126,170]. A reversed-phase column, with a mobile phase of MeOH–water (70:30) containing 100 μ*M* of EDTA, was used. The MeHg was extracted from the samples following the acid hydrolysis process of Hight and Corcoran [99] and afterwards re-extracted in an aqueous solution of cysteine with diethyldithiocarbomate to give the complex.

An entirely automatic system for the direct determination of MeHg, EtHg, PhHg and Hg(II) at levels of ng/l has been developed [171]. It is based on a pre-concentration, using solid-phase extraction (SPE) with a reversed-phase C_{18} micro-column, of the mercurial compounds formed with APDC, followed by flow-injection (FI) coupled to HPLC and CV-AAS. After the SPE of the mercury compounds, these were eluted with a mobile phase of MeOH–acetonitrile–water (38:30:32) containing APDC in an octadecilsilane (ODS) column before determination using CV-AAS. The SPE–FI–HPLC–CV-AAS method was applied to fish samples (DORM-1, CRM463 and CRM464). The samples were treated with NaCl, HCl and NaOH prior to submission to SPE.

Underivatised MeHg can be accurately and rapidly extracted from certified reference materials (DORM-1 and DOLT-2) by supercritical fluid carbon dioxide modified with MeOH (SFE). It was then quantified using LC with ED [172]. SFE does not require the use of organic liquid solvents and LC–ED does not require derivatisation of MeHg and is sensitive to reducible compounds only. Both techniques are compatible and easily coupled. The sample cartridges were threaded stainless steel tubes and granular calcium chloride ($CaCl_2$) was then added to fill the volume unoccupied by the sample. The mobile phase consists of 55% acetonitrile and 45% of a solution containing 0.08 mol/l sodium hydroxide. The practical detection limit for MeHg was $5 \cdot 10^{-8}$ mol/l when a 20-μl injection loop was used.

Finally, it is appropriate to underline the new tendencies in detection systems inter-phased to HPLC, such as a method for the structural confirmation of Hg(II) chloride and organomercury chlorides using HPLC coupled to atmospheric pressure ionization mass spectrometry applied to DORM-1 samples [173]. Also for MeHg determination in different samples (DORM-2, CRM463 and CRM464). In this case C_{18} reversed-phase columns with a mobile phase of acetonitrile–water containing 0.5 m*M* ammonium acetate to obtain a pH of 5.5 was used. The samples were treated with KCl–H_2SO_4 and subjected to distillation. Then, MeHg was complexed with sodium pyrrolidine dithiocarbamate (SPDC) at pH 6. After separation by HPLC, the compounds were reduced with $SnCl_2$ or $NaBH_4$. Different detection systems coupled to HPLC was used: ultraviolet irradiation–post-column oxidation–CV-AAS (UV–PCO–CV-AAS) [174]; high-resolution flow–mass spectrometry of plasma coupled by induction (HPF–HHPN–ICP-MS) [175]. The high recovery level obtained in the distillation process, together with the on-line pre-concentration by oxidation step (UV–PCO–CV-AAS/AFS) allowed low detection limits

(40 pg for CV-AAS and 7.5 pg for CV-AFS). On the other hand, the isotopic specific detection (HPF–HHPN–ICP-MS) permitted the isolation of low quantities of MeHg (LOD of 12.5pg) in the company of high values of Hg(II) which was the last to elute and therefore avoids interference. Also, this method did not require the use of organic solvents.

3.6. Capillary electrophoresis

An important field for application of capillary electrophoresis (CE), as an alternative to the traditional chromatographic techniques, has been the analysis and determination of metallic ions related to industrial products and environmental contaminants. Due to the magnitude of the distribution, accumulation, toxicity and decomposition processes in the different ecosystems, the speciation of heavy metals such as Hg merits special attention.

CE has showed a notable capacity for the resolution and analysis of organomercury compounds in complex samples such as fish and molluscs (tuna swordfish, mussel, clam, and cockles) [176]. The speciation of MeHg, EtHg, PhHg and Hg(II) has been performed, first of all with an extraction according to the classical procedure of Westöö, chelating the components with cysteine and separating them electrophoretically with an open silica capillary tube at 15–18 kV using 100 m*M* of sodium borate (pH 8.35) as buffer. All the species were separated in less than 12 min with a quantification limit of 10 pg.

This method of analysis has been validated with certified reference material (CRM463 and CRM464) and subjected to a quality control process [177]. According to this study, the method may be used for routine analysis of MeHg in fish and mollusc samples because of its ease of handling, high selectivity, excellent linearity, ruggedness and stability. The quantification limit was 5 pg and the detection limit was 2 pg.

A concentration technique applied to CE named ''sample stacking'' [178] has been developed to improve the detection limit. This technique is based on the injection of the sample in a low conductivity buffer or in water, followed by the application of a high voltage discharge leading to an increase in the electrical field of the analytes in the sample. This causes a high velocity migration speed to the buffer union zone where they separate according to class. A voltage of 3 kV was applied with a buffer of 0.2 *M* sodium borate (pH 8.24). The method was tested with certified reference materials (DORM-1, CRM463 and CRM464). Detection limits for MeHg of 12ng/g and quantification of 20 ng/g were obtained. Values were 10-fold smaller than in previous studies [176,177].

Recently, the CE technique has been used, substituting chelation with cysteine for sulphonate of dithizone-succinate (DzS), and complexes in the form of $[HgDzS_2]^{4-}$ and $[RHgDzS]^{2-}$ with Hg and organomercurials were obtained [179,180]. These complexes were injected in a packed capillary tube with 1% of methacryl–oxipropyl–trimethylsiloxane and a buffer of 10 m*M* of sodium acetate (pH 5). Detection limits of 20 ng were obtained and applied to certified reference materials such as DORM-1.

A comparison of CE with such well known techniques as GC–ECD and GC–MIP-AES has been described [84,138]. The results show that the injection for sample stacking, when applied to CE, improves the detection level for MeHg in biological and environmental samples, making it the best method for routine analysis.

Advances in this methodology are based on the development of an electrophoretic method for the separation and simultaneous determination of organomercury and organometallic compounds. A micellar electrokinetic chromatographic method was developed; sodium dodecyl sulphate was added to adjust the electrophoretic mobility and nitriloacetic acid was used as the derivatisation agent [181].

4. Application to samples of vegetables, meats, eggs and milk

The problem of Hg residual toxicity in different non-aqueous foods has also been the subject of interest for many years and has been widely studied by Sumino [182]. He was interested in the influence of contamination by organomercury pesticides in sprays, such as methylmercury iodide and ethylmercury phosphate in rice grains and fruits (strawberries and peppers), detecting the corresponding quantities of methyl and ethylmercury chlorides by GC–ECD.

He also studied the effect of organic mercury pesticides on rice crops, finding amounts of PhHg equivalent to 1/5–1/20 of total Hg.

Afterwards, extractions of organomercury fungicides were carried out in samples of vegetables (apples, potatoes, tomatoes, etc.) identifying them as dithizonates [183]. The samples were washed with isopropanol and cysteine (1%) and extracted with dithizone–diethyl ether (0.005%), dried with anhydrous sodium sulphate and analysed using GC–ECD. Recovery levels of 85–95% were obtained.

Phenylmercury acetate is a hazardous neurotoxic agent that may contaminate crops during spraying operations, when used as a seed dressing and herbicide. It may be separated by paper chromatography with acetone–water (70:30) as solvent system [184]. The dried chromatogram was sprayed with a 2% homogeneous of horse-liver acetone powder (source of dehydrogenase succinate) in water. This was followed after 4 min at 28°C by aqueous 0.4% 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride–aqueous 2.5% sodium succinate–aqueous 0.1% *N*-methylphenazinium methylsulphate (5:5:1) as the chromogenic reagent. The sample clean-up involves extraction of phenylmercury acetate in chloroform and concentration by evaporation. The extracts of sediments or water were screened for the presence of >900 ng amounts of phenylmercuric acetate with biodetector strips.

The method used by Westöö for extracting MeHg was used with certain modifications for the determination of MeHg in cereal samples using GC–ECD [114]. The samples were kept in benzene–formic acid (10:1) for 5 min and part of the filtrate was passed through a column prepared with silicic acid. It was eluted with benzene and cysteine acetate was added to clean the extract. Then, the aqueous phase was was acidified with HBr and the MeHg bromide was re-extracted in benzene.

A new method for isolation of MeHg was based, after previous analysis by GC–ECD, on the volatilisation of MeHg cyanide, formed in the reaction of MeHg present in the biological tissue with hydrocyanic acid from the interaction of hexacyanoferrate(II) potassium with H_2SO_4 at high temperature [185]. MeHg cyanide was retained in paper impregnated with cysteine. Once separated the MeHg was liberated with HCl and extracted with benzene. In the determination a column packed with the stationary phase of phenyldiethanolamine succinate or 4% of polyethyleneglycol succinate (PEGS) was used. The method was applied to vegetables and animals to which MeHg had been administered.

The MeHg content in a series of fungi species in a contaminated area of Germany was also studied. It was shown that these species were bioaccumulators for these compounds and were also capable of transforming inorganic Hg into MeHg. The analyses were performed using alkaline digestion with methanolic dissolution of KOH, derivatisation with $NaBEt_4$ and detection with GC–AAS [143].

Fungi have also been the subject of interest in the analysis of MeHg, EtHg, PhHg and Hg(II) using HPLC–CV-AAS. Extraction was facilitated with thiourea solution added to the samples followed by formation of the corresponding chelates with APDC [167].

Recently the sample extraction procedure proposed by Holak [163,186] has been used, but using methylene chloride as the eluent, to develop a method of determining MeHg in a series of grains, cereals, fruits and vegetables using GC–AES [30]. The samples were treated with HCl and the resulting MeHgCl was eluted in a Celite-545 column with methylene chloride. The eluent was treated with stannic chloride and the corresponding analyte was isolated from other co-extracted compounds using GC–MIP-AES. Levels of MeHg higher than 0.85 ppb were found and levels of recovery of between 70 and 114%. The detection and quantification limits found were 0.24 and 0.63 pg, respectively.

There has been less interest in the determination of organomercury compounds in other foods such as meat, eggs or milk, possibly due to their indirect relationship with these compounds and their position in the trophic chain, which makes them less susceptible to contamination with mercury species. Even then Westöö applied his method to the extraction by acid hydrolysis in samples of meat and eggs [112,113] prior to analysis using GC–ECD. Later, in a study of the determination of MeHg by GC–ECD in eggs, problems in ageing of the chromatographic columns was encountered [109].

Afterwards, Cappon and Smith [88] extracted MeHg, EtHg and PhHg as bromide, determining them by GC–ECD and applying the method to

different biological matrixes, among which were milk and cereals. This method was compared 10 years later with a new sample preparation procedure based on chelating with a solution of sodium thiosulphate and $CuCl_2$ [33]. Afterwards, the complexes were extracted with benzene for analysis using GC–ECD. It was applied to samples of milk, meat, flour and bananas.

Two methods of concentration (extraction with HCl and extraction followed by distillation), prior to separation by anionic change chromatography and CV-AAS, have been used for the quantification of MeHg in a series of reference materials of different types, among which ESB-Herring gull (eggs) and NIST-1573 (tomato leaves) [187] stand out.

5. Application to samples of natural waters

5.1. Gas chromatography

The first studies published for the separation and determination of the diverse forms of mercury in natural waters were performed with GC. Longbottom et al. [96] used GC–ECD for the determination of methylmercury in river water, after extracting the compound as chloride salt using liquid–liquid extraction. They found that strict pH control ($pH \cong 1.2$) was required to obtain the desired anion. They also concluded that extraction worked better with chloride salts than with bromide salts, as there was smaller loss due to volatilisation and that both benzene and toluene were good solvents for extraction, although due to their boiling points, benzene is preferred. In order to eliminate the different types of interference, they tried different polyester column types, finding that some may be used for up to more than six months without loss of efficiency. The level of recovery found for methylmercury in samples of river water with standard addition was 87%.

Some years later a system for the determination of MeHg in water and diorganomercurials in water [161]was presented. The MeHg was isolated from the samples as methylmercury chloride and was extracted with methylene chloride. The diorganomercurials were extracted from water either with methylene chloride or with carbon disulphide. The extracts were concentrated and analysed by GC–APAN. The interference of the other co-extracted organic molecules was eliminated observing a Hg emission line of 253.7 nm. Also, the use of this detector allows the use of methylene chloride, which significantly reduces the losses through evaporation during the preconcentration of the analyte and which are important when benzene is used as solvent [120]. The problems derived from the carbon deposits which were formed in the discharge tubes of an MIP detector were also solved [188].

In 1986 Paudyn and Van Loon [189] carried out a study for the determination of Me_2Hg, MeHg and EtHg in water samples. The compounds were extracted from water with a benzene–toluene mixture, evaporating if necessary, and analysing them with GC–AAS. For separation a column packed with Tenax is used. The detection limits of the procedure were 4 ng of Hg in Me_2Hg and MeHg and 5 ng of Hg in EtHg in 1 l of water. MeHg and EtHg were detected in natural waters (rivers, lakes and snow) in Ontario at levels of 12–45 and 7–15 ng/l, respectively, and Me_2Hg was only analysed in river water.

The previously mentioned studies used as procedure for the pre-concentration of the samples extraction with organic solvents (benzene, toluene, etc.). However, the partition coefficient for methylmercury between benzene and water was low [190] and therefore this procedure was not adequate for the analysis of samples with concentrations of less than to 0.5 ng/l.

In the quest of finding more effective extraction procedures, one was developed to determine MeHg and EtHg in natural waters [66]. The mercury compounds were concentrated by passing the water sample through a sulphhydryl cotton fibre (SCF) used as absorbent. The fibre was then eluted with a small amount of a mixture of 1 *M* HCl and 2 *M* sodium chloride. The eluent was extracted with benzene and the compounds determined by GC–ECD. The detection limit for MeHg/EtHg was in the order of 0.04 ng/l when 20 l of water was concentrated. The concentrations of MeHg found in the analysis of different samples of fresh water (lakes) were between 0.09 and 0.22 ng/l. The recovery of MeHg in water with standard-addition was notably influenced by the humus content in water and was between 42 and 68%. Those with less humus substances had the highest recovery levels. Lee and

Mowrer [100] used the same fibres (SCF) to pre-concentrate the MeHg in surface water samples. Subsequent analysis was performed using GC–ECD. Detection limits in the order of 0.05 ng/l were found in a 4-l water sample, values significantly better than those obtained with other extraction procedures [189]. The concentrations of MeHg in different samples of surface waters were between 0.08 and 0.48 ng/l.

Bloom [191] presented a technique for the determination of MeHg in aqueous samples. First, the sample was reacted with sodium tetraethylborate to convert the non-volatile MeHg in the volatile form. This was followed by the purging of the volatile product from the solution, which was collected in a graphite-carbon column at room temperature. The MeHg was desorbed from the column and analysed by GC–CV-AFS. The method allowed the simultaneous detection of labile species of Hg(II) through the formation of Et_2Hg and Me_2Hg which does not ethylate. The detection limits were close to 0.6 pg of Hg or 0.003 ng/l for 200 l of sample. The analysis of surface waters showed levels of MeHg in a range 0.02–0.10 ng/l and 0.64 ng/l when analysing a contaminated urban lake.

Accordingly, the determination of MeHg in natural waters by HS-GC–MIP has been studied, after pre-concentrating the sample containing dithiocarbamate groups and quantitatively eluting the analyte with solution of thiourea acid [149]. After this, the MeHg of the effluent was converted into iodide by the addition of sulphuric acid and iodoacetic acid, and injected into the chromatographer. The authors made a detailed study of the absorption characteristics of the resins using various procedures. The quantity of MeHg absorbed was proportional to the amount of resin used and was also closely linked to the pH value of the media.

Although the above procedures are an important advance in the process of pre-concentration of aqueous samples, they are also inconveniently lengthy. In order to find a quick and sensitive method for the analysis of mercury derivatives Rapsomanikis and Craig [192] performed derivatisation in situ of MeHg with $NaBEt_4$ in an aqueous ethanolic dissolution. They used water samples provided by the BCR from an inter-calibration exercise. No pre-treatment of the sample was made, only $NaBEt_4$ at 1% to an aliquot of the standard working dissolution to obtain derivatisation was added. After 15 min the product of the derivatisation was analysed by injecting 10 μl of the aliquot in a GC–AAS system. Comparing the results obtained with those from other methods of inter-calibration they found that the accuracy and precision of the methods were comparable without improvements and also that the procedure does not present chromatographic difficulties. The limit of absolute detection was 167 pg for MeHg.

A modification of the method proposed by Lansens [193] is that used by Emteborg et al. [194] which uses GC–MIP-AES for the simultaneous determination of species of Hg in natural waters, after concentration of the samples in a column packed with dithiocarbamate resins incorporated to a semiautomatic and closed flow injection system. The Hg species were eluted with a thiourea acid solution. After this, they were extracted in toluene as diethyldithiocarbamate and butylate complexes with Grignard's reagent. The butylate forms were injected into a GC with a gap of deactivated melted silica as the pre-column, connected to a non-polar analytic column and detected at 253.7 nm by MIP-AES. The detection limits reached were 0.5 ng/l for MeHg and EtHg and 0.15 ng/l for Hg(II) in 0.5 l samples of fresh and seawater. The procedure is not valid for analysis of water samples with high humic acid content. The authors used the same procedure in 1995 to analyse swamp water [195].

To improve the isolation and determination of organomercury species Horvat et al. [56] compared two procedures for the separation of Hg compounds in natural water samples, followed by aqueous phase ethylation, pre-collection in carbon trap, followed by GC–CV-AFS. The first isolation technique was based on the extraction of the MeHg with methylene chloride and subsequent re-extraction in water by evaporation of the solvent. The second procedure was based on the distillation of MeHg compounds. To compare the two procedures, 110 samples from different sources were analysed with a wide range of concentrations of MeHg (0.01 to 35 ng/l). Whilst extraction with solvents showed variable recovery levels, which tended to be lower with increased MeHg concentration, the average in the results obtained by distillation was higher than by extrac-

tion. Subsequent analysis of the data showed an improved agreement in the results for MeHg in concentrations lower than 1 ng/l. Samples with higher amounts of MeHg (high sulphur waters) showed better results using distillation, possibly because waters rich in sulphur are difficult to analyse using extraction with solvents.

Most of the procedures analysed until now are very tedious and require various steps. This produces loss of analyte which will affect the recovery percentages of the analysed compounds. In the last decade with the aim of reducing analysis time but maintaining or enhance detection limits, an improved method for determining total and organic Hg has been developed [196]. The organic Hg was first derivatised with $NaBEt_4$ and afterwards collected in a graphite carbon trap before transfer to an isothermally controlled GC–CV-AFS. The detection limit for MeHg in aqueous samples was 0.05 ng/l and the samples were analysed in 10 min.

Lee et al. [85] developed different pre-treatment procedures in order to separate this highly toxic species from the less toxic species of Hg. The procedures tried for pre-treatment of samples containing methylmercury are distillation, digestion with KOH–methanol and extraction with methylene chloride. Any one of the procedures may be combined with a derivatisation process by ethylation in aqueous medium with sodium tetraethylborate. Finally, the samples were determined by GC–CV-AFS. The method was applied to aqueous samples and solid matrixes.

In the last few years, advances in the determination of organomercury compounds were focussed on finding better and faster processes of pre-concentration and detection systems which led to adequate detection limits. In this sense, an analytical procedure of SPME has been described for the quantitative determination of MeHg and labile Hg(II) in aqueous matrixes [16,197,198]. The analytical procedure comprises derivatisation in aqueous phase of ionic species of Hg with sodium tetraethylborate in a sample tube and subsequent extraction with a silica fibre fitted with polydimethylsiloxane. The mercury derivates were desorbed in a splitless injector of a GC–MS system. Two different microextraction procedures were compared, SPME with HS and SPME-aqueous phase. The detection limits found for the SPME-HS procedure for 20 ml of water were 7.5 and 3.5 ng/l (as Hg) for MeHg and Hg(II), respectively, and for SPME-aqueous phase for 1.5 ml of sample were 6.7 and 8.7 ng/l (as Hg) for MeHg and Hg(II), respectively. The analysis of reference materials and river water samples without standard addition showed that the method was ideal for the determination of MeHg and Hg(II) in this type of sample although the SPME-HS procedure appears to be better as it eliminates the memory effects of Hg(II).

Similarly Mena et al. [199] proposed a new method for determination of Hg species at ng/l levels in natural waters. The mercurial derivatives (MeHg, EtHg and inorganic Hg) were pre-concentrated in a micro-column packed with sulphydryl groups incorporated in a flow-injection system. The Hg species retained were then eluted with a solution of HCl and derivatised with $NaBPh_4$ before determination by GC–MIP-AES. The detection limits for the mercurial species were 10 ng/l for MeHg and EtHg and 16 ng/l for inorganic Hg.

In order to validate new methods and test their accuracy and reliability, it is essential that certified reference materials for elements and species be used. In the case of natural waters, there are very few reliable reference materials available for the speciation of Hg. This is due to problems associated with the production of natural materials and the maintenance of the stability of Hg compounds. Given the problems associated with the maintenance of stable species in aqueous media, the possibility of reaching stability through the immobilisation of the species in a solid support was studied [200]. The stability of Hg species immobilised in sulphydryl cotton fibres (SCFs) was examined. It was found that the recovery of analyte for MeHg and Hg(II) was quantitative for up to 4 months, whilst EtHg species were stable for up to 2 months, concluding that immobilisation of mercury species in solid supports offers a further possibility of developing a new class of reference materials.

Hänström et al. [201] used a sample preparation system which was previously developed by the same authors [194] in which they greatly modified sample separation in order to inject large volumes of the sample in a GC system with packed columns connected to a GC system with capillary column and

coupled to a MIP-AES system. Thus the species can be focussed and subsequently separated in the analytical column minimising the risks of plasma extinction by solvent excess. They applied the method to the determination of Hg species in natural waters (river water) and they found that the detection limit for MeHg was 8 pg/l when injecting 50 μl of sample into the packed column, compared to 40 pg/l for direct injection of 13 μl of the sample into the capillary system GC–MIP-AES.

Ceulemans and Adams [202] developed a sensitive and automatic method free of any interference for the simultaneous analysis and speciation of methylated species of Hg, Sn, Pb and Hg(II) in water. They used a GC system with a purge and trap injector with MIP-AES (PTI-GC–MIP-AES). First, the sample was subjected to derivatisation with $NaBEt_4$, after which the ionic species were volatised and trapped in a capillary cryogenic trap where they were preconcentrated and subsequently thermally desorbed. Finally, simultaneous selective detection carried out the separation of the analytes by capillary GC–MIP-AES. The principal advantage of the technique was that on using $NaBEt_4$ as the derivatisation agent a simultaneous derivatisation of all three compounds was achieved. The detection limits found were 0.15, 0.20 and 0.60 ng/l for methylates Sn, Pb and Hg, respectively, and 2 ng/l for Hg(II). The method was applied to river and rainwater from areas heavily contaminated with organometallic compounds.

Cai et al. [203] described an analytical procedure for determination of MeHg and EtHg in aqueous samples (freshwater and seawater). The method comprised a SPE with SCF combined with a GC–AFS system using a DB-1 column for separation. Some important parameters were evaluated, such as sample pH, presence of anions and cations, concentration of dissolved organic carbon (DOC) and type and volume of eluent. The detection limits were in the order of 0.01 ng/l for both MeHg and EtHg in samples of 1 l of water. The SCF column showed a high degree of selectivity for Hg compounds and high concentration factors were achieved with improved cleaning of the samples. The procedure was applied to 21 water samples.

Hu et al. [204] developed a system for determination of MeHg which showed the added interest of being applicable to the analysis of tap water. The procedure involved the derivatisation in aqueous phase based on the formation of a more hydrophobic compound between the $MeHg^+$ and sodium tetraphenylborate (BPh_4^-) to reach MeHgPh which was extracted in dichloromethane followed by determination by GC with ion trap mass spectrometry (GC–IT-MS). The detection limits of the method for MeHg were 1 ng/ml in Hg when 100 ml of water was analysed.

Recent advances in automatic methods led to the optimisation of a totally automatic mixed system that combines the derivatisation by ethylation or generation of hydrides, pre-concentration by cryogenic trap, thermal desorption GC and detection by AAS with graphite chamber (D-CT–GC–OF-AAS) to analyse the speciation of Hg in environmental samples [205]. The automation and hyphenation leads to better reproducibility in the results. The general procedure was proposed and validated by analysis of certified reference materials for liquid samples (seawater, fresh water) and solid samples (sediments, biotissues, etc.). The detection limits of the method have been estimated as 0.1 ng/l for MeHg and Hg(II) in aqueous samples. The analysis of a sample only requires 10 min with generation of hydrides and 20 min with ethylation. The method has been applied to real samples (fresh water and seawater).

Bowles and Apte [206] evaluate a new flow-distillation technique (steam distillation) for the separation of MeHg in natural waters (fresh water, seawater and estuaries) before separation and quantification by GC–AFS. Adding APDC to the samples eliminated the co-distillation of inorganic mercury. The detection limit for this method was 0.024 ng/l of sample. The procedure was compared with distillation assisted by nitrogen, finding similar results for both methods.

5.2. High-performance liquid chromatography

One of the first studies carried out in this field [162] described a procedure for the determination of organomercury species using LC–ED. They applied the developed procedure to the analysis of fresh and salt reference waters. In order to pre-concentrate the samples they took advantage of the retention of neutral iodine complexes of the cationic species in a non-polar absorbent and subsequently desorbed them

with an organic solvent. They concluded that concentration levels of at least two-orders of magnitude higher to those obtained until now were reached and interference expected in natural water samples was eliminated.

In the 1980s and with the aim of improving sensitivity of detection new systems were developed. The main difficulty was found in the coupling of an HPLC set to sufficiently sensitive detection systems for analysis of these types of compounds. In this sense diverse systems were developed, such as HPLC–CV-AAS which was developed with aqueous standards by Fujita and Takabatake [207]. The HPLC-ICP coupling was finished by Krull et al. [208] which works on added-standard water samples. The HPLC–reductive amperometric ED of Evans and McKee [209] was finished for simultaneous determination of inorganic and organic Hg in aqueous dissolution.Also important were the HPLC–ICP-MS developed by Bushee [169] using water samples with addition of standards to validate the method. The method developed by Falter and Schöler [210] proposed an on-line coupling between LC, a PTFE coil for submission to UV radiation and CV-AAS.

Different procedures for the extraction of both organic and inorganic Hg compounds were studied [211,212]. The analytes extracted in chloroform could be directly injected by HPLC and quantified afterwards with a UV spectrometer at 254 nm. The detection limits observed were between 1.5 and 0.5 ng. The column used should have a low level of activity towards silanol groups, in order to reduce the absorption and decomposition of the Hg chelates. The second work [212] describes a procedure for extraction of mercury compounds with dithizone, subsequent separation by HPLC and UV detection. The standard Hg solution was buffered at pH 4 with an acetate buffer, transferred to a test tube and toluene and dithizone were added. It was vigorously shaken and the organic phase evaporated to dryness and was then re-dissolved in methanol. The dithizonates of Hg were separated by LC using a Spherisorb ODS-2 column, with a mobile phase of tetrahydrofuran–methanol (2:1) and an 0.05 *M* acetate buffer, pH 4 containing 50 μ*M* EDTA, to obtain a complete resolution between MeHg, EtHg, PhHg and Hg(II). The Hg chelates were determined spectroscopically at 475 nm, finding detection limits in the order of subnanograms. The method was applied to tap water, using toluene as the extraction medium. Water samples with a high copper content should be extracted with an excess of dithizone as both the copper and Hg were extracted at pH 1. A similar procedure was applied to natural waters [213].

The ability of cystine to form complexes with inorganic and organic mercury compounds is well known and frequently used in extraction procedures in biological samples [72,214]. For this reason, Sarzanini et al. [215] performed the separation of MeHg, EtHg and Hg(II) in the form of cystine complexes generated in situ for separation by ionic HPLC–CV-AAS, using a continuous flow-cell connected on-line to a reduction system with $NaBEt_4$. The cystine compounds were detected either at 210 nm in the UV region or at 253.7 nm. The proposed method was evaluated for tap water samples. The detection limits of the technique for 100 ml of water were 2, 10 and 4ng for Hg(II), MeHg and EtHg respectively.

Cammann et al. [216] performed simultaneous separation of organic ionic mercury species and led by reversed-phase HPLC with an Hypersil ODS column using pre- and on-column derivation with methylthioglycolate. Subsequent detection was performed with a UV–visible spectrophotometer at 235 nm. The mobile phase was a mixture of methanol and citrate buffer. The method was applied to rain water to demonstrate that the species under study could be determined in real samples. The method produced recovery levels of between 70 and 80% and the detection limits found were between 270 and 800 ng/l.

The behaviour of inorganic Hg(II) and organomercurials was studied by ionic HPLC using bromides of tetra-alkylammonium and sodium haloid reagents, in a mixture of methanol–water as mobile phase [217]. These sodium halides provided better peaks and lower retention times. The halides of TBABr forms a charged complex which was absorbed in UV without the need to carry out derivatisation processes with Hg(II) species, whilst small molecules such as MeHg and EtHg derivates showed better response to emission detection. The method was applied to river water.

Lately there have been many analytical procedures developed for the determination of mercury com-

pounds. Other authors developed a new technique with important improvements for the determination of MeHg, EtHg, methoxyethylmercury, ethoxyethylmercury, PhHg and Hg(II) in natural waters [210,218]. The compounds of Hg were chelated with different *N*,*N*-dithiocarbamates in order to form stable neutral compounds. These were pre-concentrated in a RP C_{18} column, and afterwards they were separated by HPLC in a similar column and determined by UV–POC–CV-AAS. The detection limit for MeHg was 0.5 ppt. This new procedure of on-line pre-concentration was applied to rain water, drinking water, surface waters and processed waters. The chelating agent that gave the best results was SPDC.

Similarly, a CV system followed by LC–ICP-MS has been described for the determination of mercury species [MeHg, EtHg and Hg(II)] in samples of water (tap water and NASS-4 of seawater as reference material) [219].

Da Silva et al. [220] proposed the study of ED using carbon electrodes, coupled to an HPLC system. For this, the Hg species were chelated off-line with SPDC at pH higher than 4 (pH between 4 and 6), optimum pH for the form of complex and necessary to obtain maximum current intensities. Following this, the mercury species were separated by LC, using as the mobile phase methanol–water (75:25) with KNO_3 as electrolytic support. The mercury complexes were detected in amperometric and coulombimetric mode at +1.15 V and +0.90 V, respectively. The detection limits of the method were between 0.16 and 2.8 ng, and it was applied to river water. In order to obtain a quantitative formation of the compounds it was necessary to add ligand excess. Better detection limits were obtained (a higher order of magnitude) with the coulombimetric detector than with the amperometric one. A study of river waters using this procedure led to recovery levels of close to 100% for the four compounds studied, MeHg, EtHg, PhHg and Hg(II), which corroborates the capacity of the method for the analysis of mercury compounds.

Finally, with the aim of improving the detection limits in the determination of MeHg and Hg(II), the effect of the composition of the mobile phase and flow speed in separation by LC has been studied, as well as the parameters that influence the CV-AAS for the separation and determination of MeHg and Hg(II) as ionic pairs [221]. The detection limits of the method proposed for Hg(II) and for MeHg were 1.13 and 1.32 ng, respectively. The procedure was applied to water from the Miño river (Galicia, Spain) to which was added between 20 and 80 ng of each compound analysed. The levels of recovery found were between 93.97% and 100% for Hg(II) and between 97.89% and 103% for MeHg.

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