Trends in Chromatography

Review

Analytical methods for mercury speciation in several matrixes: A review

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ABSTRACT

Mercury is one of the hazardous contaminants that may be present in the environment. Moreover, its toxicological effects are strongly dependent on the chemical form of the element, being the organic species the most toxic. In spite of this, the determination of mercury compounds in environmental samples, such as water, soils, sediments and biological matrixes, is of great importance. This review examines the current state of the sample treatments that have been developed, during the last 18 years (1990-2007), previous to separation and detection of mercury compounds, namely acid lixiviation, distillation, alkaline digestion and supercritical fluid extraction, as well as of the chromatographic techniques (gas chromatography and high performance liquid chromatography) used in order to separate mercury compounds and detection techniques usually coupled with them. The detection limits and the linear range achieved coupling both separation techniques with different detectors are presented, as well as the advantages and disadvantages of each one. Finally, a brief discussion over the most used detection systems is also presented.

KEYWORDS: mercury, speciation, analytical methods, gas chromatography, liquid chromatography

1. INTRODUCTION

Mercury is a heavy metal that has been studied for a long time due to its presence in the environment, related not only to rock formations (as cinnabar) but also to industrial activities. Mainly, the last ones have led to an increase in the mercury amount present in the different compartments of the environment and to its introduction into the trophic chain.

Mercury exists in a variety of chemical forms, which have different biological and environmental behaviours. It is well known that organomercury compounds are the most toxic species due to their lipophilic nature, high cellular penetration power and fast metabolization, being the ones that give cause for concern. In fact, organomercury compounds are broad-spectrum biocidal agents. It is now supposed that organomercurials can induce membrane associated oxidative stress in living organisms through different mechanisms, including the enhancement of the lipid peroxidation and intracellular generation of reactive oxygen species (ROS) [1].

Organomercury compounds can be classified into two groups: one in which mercury atom is linked to an organic radical, such as methylmercury, ethylmercury and phenylmercury; and another group in which mercury is linked to two organic radicals, such as dimethylmercury and diphenyl-mercury [2].

Methylmercury is one of the most hazardous mercury species, due to its high stability in combination with its lipid solubility and ionic properties, leading to high ability to go through membranes in living organisms. In fact, this specie presents a physiological fractionation factor, related to its turnover, of about 5-10, which

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implies that the depuration of methylmercury is several times slower than the metabolization of biomass [3]. In terms of biomagnification factor, the value corresponding to methylmercury is an order of magnitude higher than the one for inorganic mercury [3]. On the contrary, other organic mercury compounds that belong to the first group, such as phenylmercury, are decomposed rapidly in the environment.

Dimethylmercury and diphenylmercury are volatile, non polar and little water-soluble [4]. The first one is extremely lipophilic and highly volatile, and hence readily escapes into the atmosphere [4], whereas diphenylmercury in solution may undergo a photoredox decomposition induced by ligand-to-metal charge transfer excitation [5]. Moreover, dimethylmercury has high toxicity and can easily go through the skin causing the death.

Taking into account the above mentioned, as well as the great necessity of understanding the biogeochemical cycle of mercury, it is very important to develop methods for differentiating and determining organomercury species in several matrices, like waters, soils, sediments and biological material.

2. Analytical techniques used for mercury speciation

There are several analytical techniques that are used for mercury compounds determination. Some of them permit to determine separately inorganic mercury and organomercury compounds (RHg) but the last ones as a whole. Others permit to determine individually each organomercury compound. In this review, it will be only discussed those methodologies that allow the individual identification of the mercury compounds present in the samples analyzed, including sample treatment, separation technique and detection system used.

It cannot be forgotten that it is extremely important to assure a correct sampling, before performing the analysis, in order to preserve the samples integrity. Furthermore, a preconcentration step is usually required for achieving quantificable mercury amounts. However, these topics will not be discussed in this work.

2.1. Sample treatment

A number of sample treatments have been mainly developed to the quantitative release of mercury compounds from the solid sample matrix, namely acid leaching, distillation, alkaline digestion and supercritical fluid extraction (SFE). Sometimes these extractions are assisted by microwave or ultrasounds, because of both irradiation types overcome the disadvantages of conventional extraction techniques in terms of time, efficiency and solvent consumption.

Two of the most used extraction techniques for mercury speciation analysis of water samples are liquid liquid extraction (LLE) and solid phase extraction (SPE). LLE can be performed with a suitable organic solvent, or a mixture of organic solvents. Low ionic organomercury compounds are easily extracted using a suitable organic solvent, whereas the need for adding a complexing agent increases with increasing ionic character of mercury specie. Several works also report the use of the SPE or solid-phase microextraction (SPME) to extract and/or preconcentrate the analytes. In fact, these techniques are rapidly growing in popularity, being sulphydryl cotton fibers and complexing resins the most used sorbents.

Table 1 shows relevant sample treatments reported for mercury speciation in several matrices.

All of these sample treatments have some drawbacks associated, such as incomplete extractions, an insufficient derivatization or distillation and the existence of interferences in the detection step, which must be strictly controlled. Additionally, it must be considered that it is difficult to estimate the mercury recovery for the extraction procedures using samples spiked because the methylmercury added is not always bound the same way as the one naturally occurring in the sample [78, 79].

2.1.1. Acid leaching

The main objective of this method is to separate inorganic mercury compounds from the organic ones (RHg), and to separate them from other sample components, such as proteins and humic matter, which could interfere in the detection step [80]. One of the most cited methodologies for mercury speciation is the classical Westöö method, which consists in the use of a strongly acidic hydrochloric solution with the aim of liberating mercury species from thiol groups of proteins [81]. The most of the missing organo-mercury compounds remains in the sample

Table 1. Sample treatments applied to different matrixes in order to perform mercury speciation.

Matrix	Previous Treatment	Treatment to obtain RHgX	Organic Solvent	Aqueous Solution	Separation Method	References
Water	Dithiocarbamate resin microcolumn + thiourea elution + NaOH + borate buffer	Diethyldithiocarbamate	$CH_3C_6H_5$		Butylation-$GC_{capillary}$	[6]
	KCl[1]	HCl	CH_2Cl_2	Water	Ethylation-GC_{packed}	[7]
	KCl[2]	H_2SO_4[3]+ Distillation			Ethylation-GC_{packed}	[7]
		HCl	$CH_3C_6H_5$	$Na_2S_2O_3$	HPLC	[8]
	SCF[4]	KBr/$CuSO_4$ in H_2SO_4	CH_2Cl_2		$GC_{capillary}$	[9]
		KBr in H_2SO_4 + Distillation			Ethylation-GC_{packed}	[10]
		KCl in HCl	CH_2Cl_2	Water	Ethylation-GC_{packed}	[10]
		Diethyldithiocarbamate (pH 9.5)	$CHCl_3$		HPLC	[11]
	SCF[4]	KBr/$CuSO_4$ elution	CH_2Cl_2		$GC_{capillary}$	[12]
	SPE[5] + Acidified thiourea (pH 9.22) + NaCl + NaOH	Diethyldithiocarbamate	Hexane		Butylation -$GC_{capillary}$	[13]
	SPE[5] + NaOH + borate buffer + NaCl	Diethyldithiocarbamate	Hexane		Butylation-$GC_{capillary}$	[14]
	SCF[4]	HCl		NaOH-CH_3COONa at pH =2	Derivatization-$GC_{capillary}$	[15]
		KCl in H_2SO_4 + Distillation with vapor			Ethylation-GC_{packed}	[16]
	pH correction at 4.0				Ethylation-$GC_{multicapillary}$	[17]
	Acidification (HNO_3 conc.)				Hydride-GC_{packed}	[18]
	Diethyldithiocarbamate (pH 4.5) + C60 fullerene column	Elution with ethylacetate + derivatizing agent			tetra-*n*-propylborate Derivatization - $GC_{capillary}$	[19]
	LLLME[6] (donor phase pH = 6)				HPLC	[20]

Table 1 continued..

Sediment		HCl	$CH_3C_6H_5$	$Na_2S_2O_3$ buffered with ammonium acetate	HPLC	[21]
		Urea+$CuSO_4$+HCl	$CH_3C_6H_5$	$Na_2S_2O_3$ + $CuCl_2 \rightarrow CH_3C_6H_5$	$GC_{capillary}$	[22]
	Acetic acid (Ultrasounds)				Hydride-$GC_{capillary}$	[23]
		HCl	$CH_3C_6H_5$	$Na_2S_2O_3$ buffered with ammonium acetate (pH=5.5)	HPLC	[24]
		HCl	$CH_3C_6H_5$		Alkylation-$GC_{capillary}$	[24]
	Citrate buffer (pH=2) + dithizone in $CHCl_3$	$NaNO_2$ in $HCl/H_2SO_4/NaCl$	$CHCl_3$	$Na_2S_2O_3$ buffered with ammonium acetate	HPLC	[25]
		KOH/MeOH			Ethylation-GC_{packed}	[26]
	KOH/methanol	KCl + H_2SO_4 + Distillation			Ethylation-GC_{packed}	[26]
		HCl			Ethylation-GC_{packed}	[26]
		HCl + Distillation			Ethylation-GC_{packed}	[26]
		KCl + H_2SO_4 + Distillation			Ethylation-GC_{packed}	[26]
		$CuSO_4$/KBr in H_2SO_4	CH_2Cl_2	$Na_2S_2O_3$ + $CuCl_2$ $\rightarrow CH_2Cl_2$	$GC_{capillary}$	[27]
		Citrate buffer (pH=2) + dithizone in $CHCl_3$ + $NaNO_2$	$CHCl_3$	$Na_2S_2O_3$ + NH_4OAc	HPLC	[8]
	NaOH				Ethylation-$GC_{capillary}$	[28]
	Citrate buffer (pH=2) + dithizone in $CHCl_3$	$NaNO_2$ in a H_2SO_4/NaCl	$CHCl_3$	$Na_2S_2O_3$ + NH_4OAc	HPLC	[29]
	-	HCl	$CH_3C_6H_5$	$Na_2S_2O_3$ + NH_4OAc	HPLC	

Table 1 continued..

	Citrate buffer + dithizone in $CHCl_3$ + $NaNO_2$	$CHCl_3$+ acid mixture	$Na_2S_2O_3$	HPLC	[30]
	$CuSO_4$/KBr in H_2SO_4	CH_2Cl_2	$Na_2S_2O_3$ + $CuCl_2$ → CH_2Cl_2	$GC_{capillary}$	[9]
SFE[7]		$CH_3C_6H_5$		Butylation-$GC_{capillary}$	[31]
	KCl in H_2SO_4 + Distillation + Borate buffer + diethyldithiocarbamate	$CH_3C_6H_5$		Butylation-$GC_{capillary}$	[31]
KOH-MeOH	HCl	CH_2Cl_2	Ultra-pure water	Ethylation-GC_{packed}	[32]
KOH/MeOH + Ultrasounds	H_2SO_4 + $CuSO_4$ + KBr	$CH_3C_6H_5$	Cysteine → Benzene+ $CuSO_4$ +KBr	$GC_{capillary}$	[33]
HCl + $CH_3C_6H_5$ + Microwave	Cysteine acetate; HCl	$CH_3C_6H_5$		$GC_{capillary}$	[34]
HNO_3[8] + Microwave				Ethylation-GC_{packed}	[35]
	NaCl in H_2SO_4 + Distillation			HPLC	[36]
	KCl + H_2SO_4 + Distillation			HPLC	[37]
	H_2SO_4+NaCl+$CuCl_2$+ Ultrasounds + Distillation under vacuum			Hydride-GC_{packed}	[38]
HNO_3+ Microwave				Ethylation-$GC_{multicapillary}$	[39]
HNO_3+ Microwave				Ethylation-GC_{packed}	[18]
HNO_3 + Microwave				Ethylation-$GC_{multicapillary}$	[40]
HNO_3 or HCl + Microwave				Ethylation-GC_{packed}	[41]
	HCl	$CH_3C_6H_5$	Cysteine → $CH_3C_6H_5$	$GC_{capillary}$	[42]
	HCl	$CH_3C_6H_5$		$GC_{capillary}$	[42]

Table 1 continued..

		H_2SO_4 + NaCl	$CH_3C_6H_5$	$Na_2S_2O_3$	Hydride-$GC_{capillary}$	[42]
	HNO_3 + Microwave				Ethylation-GC_{packed}	[42]
		H_2SO_4 + KCl + distillation			Ethylation-GC_{packed}	[42]
		H_2SO_4 + NaCl + distillation[9]		Pyrrolidinedithio carbamate	HPLC	[42]
		HCl	$CH_3C_6H_5$	Thiosulphate + acetate buffer	HPLC	[42]
	SFE[7]		$CH_3C_6H_5$		Butylation-$GC_{capillary}$	[42]
	KOH-MeOH	HCl	CH_2Cl_2	Ultra-pure water	HPLC	[43]
	KOH-MeOH + Microwave	HCl	CH_2Cl_2	Ultra-pure water	HPLC	[44]
Biological material		HCl	$CH_3C_6H_5$	$Na_2S_2O_3$	Ethylation-GC_{packed}	[45]
		HCl + Borate buffer + diethyldithiocarbamate	$CH_3C_6H_5$		Butylation-$GC_{capillary}$	[46]
	KOH-MeOH + Ultrasounds				Ethylation-GC_{packed}	[47]
	$(CH_3)_4NOH$	HCl + dithizone in $CHCl_3$ + $NaNO_2$ in HCl/H_2SO_4/NaCl	$CHCl_3$	$Na_2S_2O_3$ buffered with NH_4OAc	HPLC	[25]
		NaCl + HCl + Ultrasounds			HPLC	[48]
		HCl + Ultrasounds	$CH_3C_6H_5$	$Na_2S_2O_3$	HPLC	[49]
		3% KBr + 3 mol dm^{-3} HBr + $CuSO_4$	$CH_3C_6H_5$	Mobile phase	HPLC	[50]
		HCl + Ultrasounds			Hydride-$GC_{capillary}$	[51]
	Acetone + Water	HCl	$CH_3C_6H_5$		$GC_{capillary}$	[52]
		$CuSO_4$/KBr in H_2SO_4	CH_2Cl_2	$Na_2S_2O_3$ + $CuCl_2$ $\rightarrow$ CH_2Cl_2	$GC_{capillary}$	[9]
	KOH (Ultrasounds) + SPME[10]				Ethylation-$GC_{capillary}$	[53]

Table 1 continued..

$NaOH + H_3PO_4$ (pH 6.1-6.4)	Diethyldithiocarbamate (pH 5)	C_6H_{14}		Penthylation-$GC_{capillary}$	[54]
KOH-MeOH	CH_2Cl_2 + HCl	CH_2Cl_2	Water	Ethylation-GC_{packed}	[32]
	CH_3CN + pyrrolidine dithiocarbamate (pH 5.5)			HPLC	[55]
	NaCl in H_2SO_4 + Distillation			HPLC	[36]
	KCl + H_2SO_4 + Distillation			HPLC	[37]
	HCl	Benzene	Cysteine → Benzene	$GC_{capillary}$	[56]
$(CH_3)_4NOH$ + Microwave				Hydride- GC_{packed}	[57]
	HCl	Diethyldithio carbamate in $CH_3C_6H_5$		Butylation-$GC_{capillary}$	[58]
Acetone + $CH_3C_6H_5$	HCl	$CH_3C_6H_5$		$GC_{capillary}$	[59]
	HCl + Celite + SFE[7]	$CH_3C_6H_5$		$GC_{capillary}$	[59]
$(CH_3)_4NOH$ + Microwave				Ethylation-$GC_{multicapillary}$	[39]
$(CH_3)_4NOH$ + Microwave				Ethylation-$GC_{multicapillary}$	[60]
	NaCl + HCl (pH=4)			HPLC	[61]
$(CH_3)_4NOH$ + Microwave				Ethylation or Hydride - GC_{packed}	[18]
$(CH_3)_4NOH$ + Microwave				Ethylation - $GC_{multicapillary}$	[40]
KOH	KBr/$CuSO_4$	CH_2Cl_2	$Na_2S_2O_3$ → CH_2Cl_2	$GC_{capillary}$	[62]
CH_3COOH + $NaBPH_4$ + $CH_3C_6H_5$ + Microwave			$CH_3C_6H_5$; Alumina column → $CH_3C_6H_5$	$GC_{capillary}$	[63]

Table 1 continued..

KOH-MeOH + microwave	HCl	CH_2Cl_2	Ultrapure water	HPLC	[64]
	NaCl + HCl			HPLC	[65]
HCl - Microwave				Phenylation – Headspace SPME - $GC_{capillary}$	[66]
Protease type XIV				HPLC	[67]
$(CH_3)_4NOH$ followed by pH adjustment				HPLC	[68]
KOH				Propylation – Headspace SPME - $GC_{capillary}$	[69]
H_2O/CH_3OH containing 2-mercaptoethanol				HPLC	[70]
$(CH_3)_4NOH$ + Microwave	Cu^{2+} (pH 4)		Tetrapropylborate/ KOH → *n*-heptane	$GC_{capillary}$	[71]
Ultrasounds + detergent solution (pH 4.5)				Ethylation – Headspace SPME - $GC_{capillary}$	[72]
	HCl + sonication			HPLC	[73]
	KBr-$H_2SO_4/CuSO_4$	$CH_3C_6H_5$	$Na_2S_2O_3$	HPLC	[74]
	L-cysteine-HCl-H_2O + heating			HPLC	[75]
HCl + Ultrasounds – LLLME[6] (donor phase pH: 1 mol dm^{-3} HCl)				HPLC	[20]
	Microwave-assisted extraction + HCl + NaCl			HPLC	[76]
$(CH_3)_4NOH$ + Microwave					[77]

[1]Applied to fresh water, being omitted in salt water; [2]Applied to fresh water, being omitted in salt water or in samples pre-acidified with HCl; [3]Omitted in samples pre-acidified with HCl; [4]Sulphydryl cotton fiber; [5]Solid phase extraction; [6]Liquid-liquid-liquid microextraction (toluene – organic phase; $Na_2S_2O_3$ – acceptor phase); [7]Supercritical fluid extraction; [8]Corresponds to the best result obtained using several extractants; [9]Subsequently, it is buffered to pH 6 with ammonium acetate; [10]Solid phase microextraction.

because either it is decomposed in the presence of strong acids or is bound so strongly that it could not be extracted using weak acids [24], as occurred in sediments rich in organic matter [27, 82]. In biological samples, the usage of concentrated acids can originate mercury losses by evaporation, methylmercury degradation to inorganic mercury and insufficient dissolution of the sample originating low methylmercury recoveries.

RHgCl compounds are afterwards extracted from the aqueous phase into an organic solvent. The losses occurred during the extraction into the organic phase can also be explained by the decomposition of methylmercury, because inorganic mercury is not easily extracted in the organic phase [24]. RHgCl compounds can be co-extracted with a cysteine solution. In gas chromatography (GC), it is necessary to extract again RHg species into an organic solvent. Owing to this, the aqueous solution is acidified in order to break the cysteine-RHg complexes, being RHg compounds again extracted into an organic solvent, such as toluene or benzene.

Some works refer the use of nitric acid [18, 35, 39, 40, 42], nitric acid plus hydrogen peroxide [83], or acetic acid [23], instead of hydrochloric acid. The acid leaching can be assisted by ultrasounds [23] or microwaves irradiation [18, 35, 39, 40, 42, 66, 84], shortening the sample treatment time.

Sequential extraction schemes allow classifying mercury species based on their environmental mobility and/or toxicity for on-site screening purposes [85]. The alkyl mercury species and soluble inorganic mercury species that majory contribute to potential mercury toxicity in soils are extracted by an acidic ethanol solution (2% HCl+10% EtOH) as mobile and toxic species. Then, inorganic mercury species remaining in soil are further divided into semi-mobile and nonmobile sub-categories by sequential acid extractions. The semi-mobile species include mainly elemental mercury and mercury-metal amalgams. The nonmobile Hg species mainly include mercuric sulfide (HgS) and mercurous chloride (Hg_2Cl_2).

2.1.2. Distillation

Several works refer the use of distillation, following the scheme represented in Figure 1, as a way to concentrate the organomercury species [7, 10, 26, 36, 37, 86].

Distillation requires: i) the addition to the sample of potassium chloride and sulphuric acid [7, 26, 37], hydrochloric acid [26], potassium bromide and sulphuric acid [10] or sodium chloride and sulphuric acid [36, 87]; ii) heating at 140ºC [87], 145°C [7, 26] or 180°C [36, 37]; iii) a flux of nitrogen [7, 26, 36, 37, 87] or water vapour [16]. The combination of the acidification with the halide addition permits the dissociation of the organomercury complexes and the formation of volatile halides [16].

Distillation is normally realized until 80-85% of the sample has been distillated [7, 10], in order to prevent the volatilization of compounds that can suffer decomposition at the end of the distillation.

Although distillation is efficient to separate methylmercury from inorganic mercury, in some sediment samples, it was observed the presence of the last mercury specie in the distilled [26]. The temperature and distillation rate are important

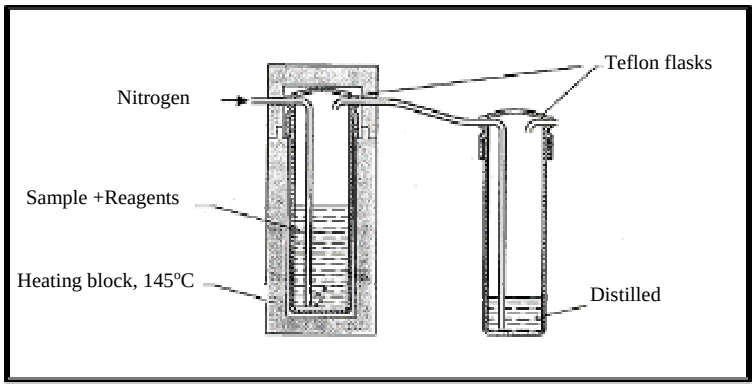


Figure 1. Distillation apparatus [26].

parameters that must be conveniently controlled. If high temperatures are used (> 150ºC), methylmercury recoveries are low and not reproducible [7]. Moreover, high distillation rates can cause the passage of the solution, present in the sample flask, to the collector flask of the distilled [26]. On contrary, if the temperatures are very low, the distillation rates can be very slow [7].

The formation of methylmercury during distillation from inorganic mercury was also reported by several authors [42, 88-90]. The magnitude of this accidental methylation [90] increases linearly with total mercury content, being higher in the presence of humic matter whereas it is not observed in the presence of biological material. However, the artifact formation can be avoided interrupting distillation when approximately 85% of the liquid has been distilled [42, 90].

2.1.3. Alkaline digestion

This method is also used for the treatment of solid matrixes, involving the addition of potassium hydroxide in methanol [26, 32, 33, 43, 44, 47, 64] or tetramethylammonium hydroxide [57, 60, 68, 71, 77, 91]. Moreover, some alkaline digestions are also assisted by ultrasounds [33, 47] or microwave irradiation [44, 57, 60, 64, 71, 77, 91, 92] in order to short sample treatments and increase the extraction efficiency.

Mercury species transformations occur during biological tissues pre-treatment. Thus, methylation of inorganic mercury probably takes place mainly during and after pH adjustment and it decreases after prolonged treatment with tetramethyl ammonium hydroxide. Therefore, to minimize abiotic methylation when pH adjustment is required, it is recommended to proceed after samples have been treated with tetramethyl ammonium hydroxide for 24 h [68].

Some authors [26, 93] have published that this method liberates quantitatively methylmercury from sediments. However, [88-91, 94] it is also mentioned the possibility of occurring a positive error in methylmercury determination in sediments, when performing alkaline digestion followed by ethylation, due to the formation of this mercury compound.

2.1.4. Supercritical fluid extraction

In 90's decade, several works involving supercritical fluid extraction have been reported in mercury speciation [31, 59]. The main advantage of this extraction method is linked to the supercritical fluids properties, such as density, viscosity and solute diffusivity, which can be controlled through pressure and temperature variations [59]. It permits to accelerate the extraction process and reduce the quantity of the solvents used [59, 95].

The popularity of supercritical fluid extraction has been increasing, as stated by Hill [96], due to the high efficiencies obtained under mild conditions. However, it was observed a low mercury recovery (58 ± 6%) when the certified sediment CRM 580 was spiked with methylmercury [42].

2.2. Separation and detection

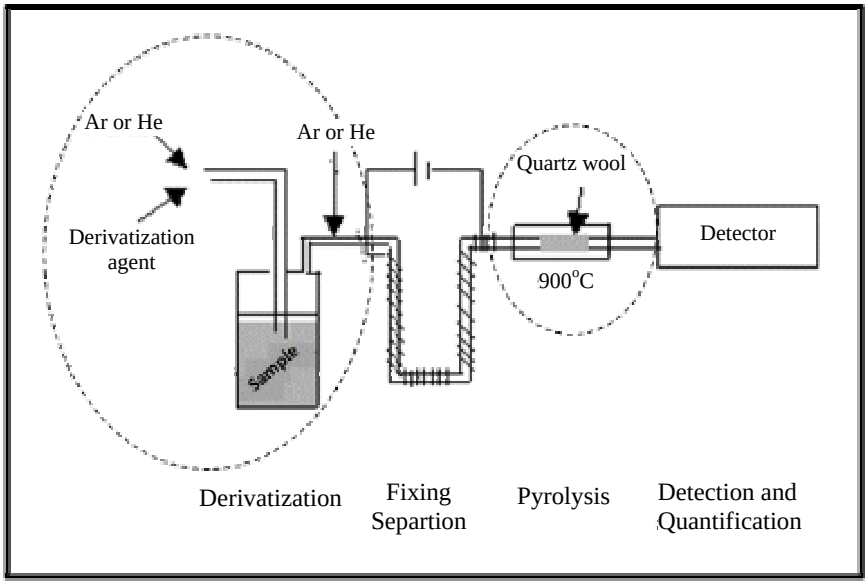
The most widely used separation technique for organomercury compounds is gas chromatography (GC). However, a significant number of works uses high performance liquid chromatography (HPLC) to perform mercury compounds separation. Although other alternative techniques, such as supercritical fluid chromatography (SFC) [97] and capillary electrophoresis [98, 99], have been also applied to organomercurials separation, only GC and HPLC will be discussed in the next section because their great popularity.

2.2.1. Gas chromatography

The speciation methods based on the use of GC as separation technique involve some or all of the following analytical steps: derivatization, chromatographic separation, pyrolysis, detection and quantification (Figure 2).

Several GC works [9, 22, 27, 34, 52] are related with the direct determination of organomercury compounds in the organic extracts previously obtained during the sample treatment. Unfortunately, the polarity of monoalkylated mercury compounds can induce the irreversible adsorption on active sites of the stationary phase of the chromatographic column, causing undesirable effects, like peaks widening and retention time variability. This is due to the stationary phase stops being inert with usage and, therefore, dismutation reactions can occurr [40]. These problems affect directly the efficiency of the separation method by decreasing the analyses reproducibility.

The reduction of these effects requires a column conditioning treatment [9, 18]. Therefore, it is



Note: The components included in the dotted area may be present or not.

Figure 2. Schematic diagram of GC (packed or capillary columns) used for mercury compounds speciation.

necessary to inject regularly $HgCl_2$ in toluene [22, 34, 52, 100], dichlorodimethylsilane in toluene [101], dichloromethane [12], bromic acid in a methanolic solution [56] or hexamethyldisilane [18]. However, the last procedure can induce methylmercury formation [41], depending its production on the quantity added of the silanizing agent, as well as on the silanizing time [41].

On the other hand, some authors [10, 16, 26] perform mercury compounds derivatization before proceeding to their separation by GC. Derivatization allows the transformation of ionic species (polar character), into non polar forms. These last forms are more volatile than ionic ones and, furthermore, they do not decompose in the chromatographic column due to their higher thermal stability [54, 102-104].

a) Derivatization methods

The most used derivatization method is ethylation, which consists in the conversion of mono-alkylated mercury compounds in their dialkylated derivatives, using sodium tetraethylborate ($NaBEt_4$) [7, 17, 18, 26, 32, 35, 40, 45, 47, 53, 104, 105]. This reacts with inorganic mercury and methyl-mercury, forming diethylmercury (Et_2Hg) (1) and ethylmethylmercury (MeEtHg) (2), respectively, [18, 35, 45, 47] according to the following reactions:

$$Hg^{2+} + 2\ NaBEt_4 \rightarrow Et_2Hg + 2'BEt_3' + 2Na^+ \quad (1)$$

$$MeHg^+ + NaBEt_4 \rightarrow MeEtHg + 'BEt_3' + Na^+ \quad (2)$$

where "BEt_3" or $B(C_2H_5)_3$ represents an unstable specie, which reacts with air and water.

Another derivatization agent is sodium tetrahydroborate ($NaBH_4$) [18, 23, 51]. This compound converts methylmercury into a volatile methylmercury hydride (MeHgH) (3) [18, 51, 106] and reduces inorganic mercury to elemental mercury (Hg^0) (4) [18, 51, 106]. Furthermore, dimethyl-mercury is purged unchanged, when it is present.

$$MeHg^+ + NaBH_4 + 3H_2O \rightarrow MeHgH + 3H_2 + H_3BO_3 + Na^+ \quad (3)$$

$$Hg^{2+} + 2NaBH_4 + 6H_2O \rightarrow Hg^0 + 7H_2 + 2H_3BO_3 + 2Na^+ \quad (4)$$

The volatile mercury species are trapped in the column, which is immersed in liquid nitrogen. Subsequently, the column is gradually heated, being the trapped mercury species sequentially eluted on the basis of their boiling points.

Some works refer the use of other less-known derivatizing agents, such as sodium tetraphenyl-borate [15, 63, 107] or sodium tetra (*n*-propyl) borate [69, 91, 108, 109]. Grignard reagents have also been used for derivatization, such as buthyl-magnesium chloride in tetrahydrofurane [13, 14, 31, 42, 58] or *n*-penthylmagnesium bromide [54].

The Grignard reagents are added to substitute the polar ligands present in mercury compounds, for example, by buthyl groups [110]. Elemental mercury does not react significantly with the Grignard reagent, but methylmercury chloride and mercury chloride react rapidly with it, forming the corresponding buthyl derivatives [110]. The derivatization involving Grignard reagents requires the existence of a non aqueous and completely dry medium [104, 111] because these reagents are water sensitive [108]. Therefore, it is necessary a previous extraction step into a non polar organic phase [104].

In relation to these derivatization methods, the hydride generation method, involving $NaBH_4$, has the advantage of generating rapidly hydrogen, which can instantaneously purge the formed elemental mercury and methylmercury hydride, as well as unchanged dimethylmercury and diethylmercury, towards the chromatographic column [18, 80]. The compounds purge is slower and less efficient using ethylation method due to the necessity of a carrier gas. However, the $NaBH_4$ used for the hydride generation can form radicals that will promote the breakdown of the carbon-mercury bonds present in organomercury compounds, inducing their reduction to elemental mercury [104], in the presence of oxygen or the high nitric acid concentrations usually used in the sample digestion.

When ethylation is used as derivatization method, it is not possible to distinguish between inorganic mercury and ethylmercury [12, 54, 62, 107], whose occurrence has been reported in soil and sediment, and the costs associated seem to be higher than the ones associated with phenylation [107]. Moreover, ethylation reaction is very sensitive to pH and to the excess of ions in solution [72]. If the ethylation conditions are not optimized, dismutation reactions can occur. For example, methylmercury can originate elemental mercury and dimethylmercury [47], or inorganic mercury can induce elemental mercury formation for high pH values [41]. The reagent quality is another important aspect to taking into account. In fact, high inorganic mercury concentrations in $NaBEt_4$, used in ethylation method, can generate the formation of artifacts [26, 40, 41, 62].

During propylation, artifact formation of methylmercury, ethylmercury and diethylmercury from inorganic mercury was also observed [109], correlating positively with the amount of inorganic mercury present in the derivatization solution. Nevertheless, the formation of artifact methylmercury from inorganic mercury using $NaBPr_4$ seems to be poorly reproducible [109]. The artifact formation can be due to the occurrence of complicated side reactions by $NaBPr_4$, such as alkyl cleavage and rearrangement.

In relation to derivatization methods promoted by Grignard reagents, it must be referred that the formed by-products have higher boiling temperatures than the ethylated forms or the hydrides obtained using the other derivatization methods. It originates higher elution times and problems in the volatilization of species with high molecular weights [104].

b) Mercury compounds separation

Mercury compounds separation has been performed in several types of chromatographic columns:

- Packed [18, 32, 35, 42, 45, 47, 80, 105, 106];
- Capillary [9, 13-15, 24, 27, 28, 31, 34, 42, 54, 59, 62, 63, 69, 72, 91, 100, 102, 104, 107];
- Multicapillary [17, 39, 40].

These last columns result from the joining of several capillary columns in parallel allowing the injection of a higher sample volume and, therefore, the decreasing of the minimal detectable concentration [17].

When packed columns are used for the separation of different mercury species, it is usual to perform a previous fixation of the compounds in that column [18, 35, 47, 80, 106] or in other column installed previously to the one used for the separation [7, 26, 105]. The fixation is usually done at low temperatures, using liquid nitrogen (cryogenic trapping) [18, 35, 47, 80, 106], or at ambient temperature [32, 105]. Several works, that involve capillary columns for mercury compounds separation, also refer a previous fixation in packed [104] or capillary [40] columns, installed before the one responsible for the mercury species separation. A relevant aspect to taking into account before fixing the compounds is the necessity of removing the water present; otherwise it can occur either water freezing, blocking the chromatographic column [106], or the plasma extinction, if this is present in the detection system, due to water vapour release during the purge [104]. The most used water

removal systems are the Nafion membranes [17, 40, 106] or a condenser with ethanol at -15°C [104].

The stationary phase of the packed chromatographic columns used for the fixation/separation of mercury compounds is methylsilicone (OV or SP-2100 designation) [7, 42, 105] on a solid support of Carbotrap [26, 105], Tenax [104, 105], and Chromosorb W or G [18, 26, 42, 45, 47, 57, 80, 104, 106]. On the other hand, the most used stationary phases of the capillary columns used for the fixation and/or separation of mercury species are:

- 100% dimethyl polysiloxane on BP-1 [34, 42, 59], SE-30 [17, 40], OV-101 [14], HP-1 [15, 54, 100, 107, 109] and DB-1 [9, 12, 27, 31, 42, 62, 107];
- 100% methyl silicone fluid on SP-2100 [23]. The polymer of this stationary phase has identical polarity than the previous;
- 5% phenyl-95% methylsiloxane on HP-5 [31, 100], DB-5 [72], SE-54 [17], HP-5-MS [19], DB-5ms [63, 66, 69] and CP-SIL 8CB [40, 42, 91];
- 14% cyanopropyl-phenyl-methylpolysiloxane on DB-1701 [24, 102];
- 6% cyanopropylphenyl-94% dimethylpolysiloxane on DB-624 [13];
- 35% diphenyl-65% dimethyl polysilphenylene-siloxane, as for example on the SPB-608 [42].

The chromatographic column is then gradually heated, being the trapped mercury species brought to the detector using a carrier gas, like argon [40, 72, 105-107], helium [7, 9, 12-15, 18, 19, 24, 26, 27, 31, 35, 42, 47, 54, 62, 63, 66, 69, 80, 91, 100, 102, 104, 105, 107] or nitrogen [31, 34, 42, 45].

c) Pyrolysis

The pyrolysis implies the formation of elemental mercury by heating at high temperatures (800 to 900°C). If plasmas are used after mercury compounds separation, the pyrolysis will not be necessary because the plasmas are able to atomize and excite a great quantity of gases [102, 104]. Nevertheless, the pyrolysis, as the way of elemental mercury production, require to achieve temperatures of 800°C [9, 12, 13, 18, 27, 35, 42, 45, 72, 105], 825°C [80], 830°C [47], 850°C [62] or even 900°C [7, 26, 106].

Some GC works refer the possibility of organomercury species decomposition due to the contact with hot surfaces [12, 105]. This decomposition can occur when thermal desorption is performed in packed columns of Carbotrap [12, 105] or into the chromatographer injectors, which are at very high temperatures [12]. Thus, parameters related to quality and porosity of the column material, as well as its heating temperature, have extreme importance in GC works and should be carefully checked.

Furthermore, it is important to mention that the transfer lines between column and detector should be as short as possible [112] and heated in order to prevent the analyte condensation [96, 112].

d) Detection techniques

After GC separation, the most used detection techniques for mercury compounds identification and quantification are the following:

- Atomic absorption spectrometry (AAS) [13, 18, 23, 24, 35, 42, 45, 47, 57, 72, 80];
- Atomic fluorescence spectrometry (AFS) [7, 9, 12, 26, 27, 32, 62, 105-107];
- Atomic emission spectrometry (AES) [63, 100, 107];
- Microwave induced plasma-atomic emission spectrometry (MIP-AES) [14, 15, 17, 31, 39, 42, 54, 66, 100, 104];
- Glow discharge-atomic emission spectrometry (GD-AES) [58];
- Plasma emission detection (PED) [28];
- Electron capture detection (ECD) [34, 42, 56, 59, 100];
- Mass spectrometry (MS) [19, 69, 71, 91, 107];
- Inductively coupled plasma-mass spectrometry (ICP-MS) [40, 62, 102, 109, 113].

2.2.2. Liquid chromatography

Liquid chromatography (LC) is undoubtedly another separation technique used for mercury speciation [114]. In particular, high performance liquid chromatography (HPLC) offers several advantages over gas chromatography (GC). One of the main advantages is that mercury compounds can be separated at ambient temperature without derivatization. Thus, the time required for analysis, as well as the potential analyte losses, are reduced. Furthermore, HPLC offers great versatility; a large variety of stationary phases is available for this technique and both the mobile and stationary phases can be simultaneously varied in order to achieve good separations. Reversed phase

techniques, involving an ion-pairing agent, and ion-exchange chromatography are widely used for the separation of inorganic and organic mercury compounds.

The speciation methods based on the use of HPLC as separation technique involve some or all of the following analytical steps: preconcentration, chromatographic separation, oxidation, reduction, detection and quantification (Figure 3).

a) Preconcentration

Sometimes, the analysis of environmental samples, like water samples, involves the incorporation of a preconcentration step previous to chromatographic separation in order to achieve quantifiable mercury quantities. The retained analytes are then eluted onto the analytical column. Although different approaches have been proposed for mercury determinations at trace levels, the recent developments in the field of mercury compounds preconcentration from several samples are focused to on-line solid phase extraction (SPE) in flow injection (FI) systems by the use of a packed minicolumn. A diversity of combinations between solid sorbent and complexing agent has been employed for preconcentration of both inorganic mercury and organomercury compounds, as described in Table 2.

Several complexing solid sorbents have been applied to on-line preconcentration of mercury species from natural water samples using different detection techniques. Thus, sulphydryl cotton permitted to retain methylmercury, ethylmercury and inorganic mercury, which were then eluted with 3 mol dm^{-3} hydrochloric acid. Emteborg *et al.* [129] incorporated a microcolumn of dithiocarbamate resin in a FI system to preconcentrate mercury species, being used acidic thiourea as eluent. On the other hand, mercury compounds were preconcentrated on a column containing 2-mercaptobenzimidazol loaded on silica gel and then quantitatively eluted with 0.05 mol dm^{-3} potassium cyanide or 2 mol dm^{-3} hydrochloric acid for inorganic mercury or methylmercury, respectively.

Several authors have also proposed off-line enrichment of mercury species on complexing resins. Emteborg *et al.* [130] introduced in natural water samples the complexing resin, prepared by immobilization of dithiocarbamate functional groups on macroporous hydroxyethyl methacrylate spheres, and the suspension was stirred, whereafter the samples were filtered. Mercury species were extracted with acidified thiourea. The determination of inorganic mercury and organomercury compounds was also carried out

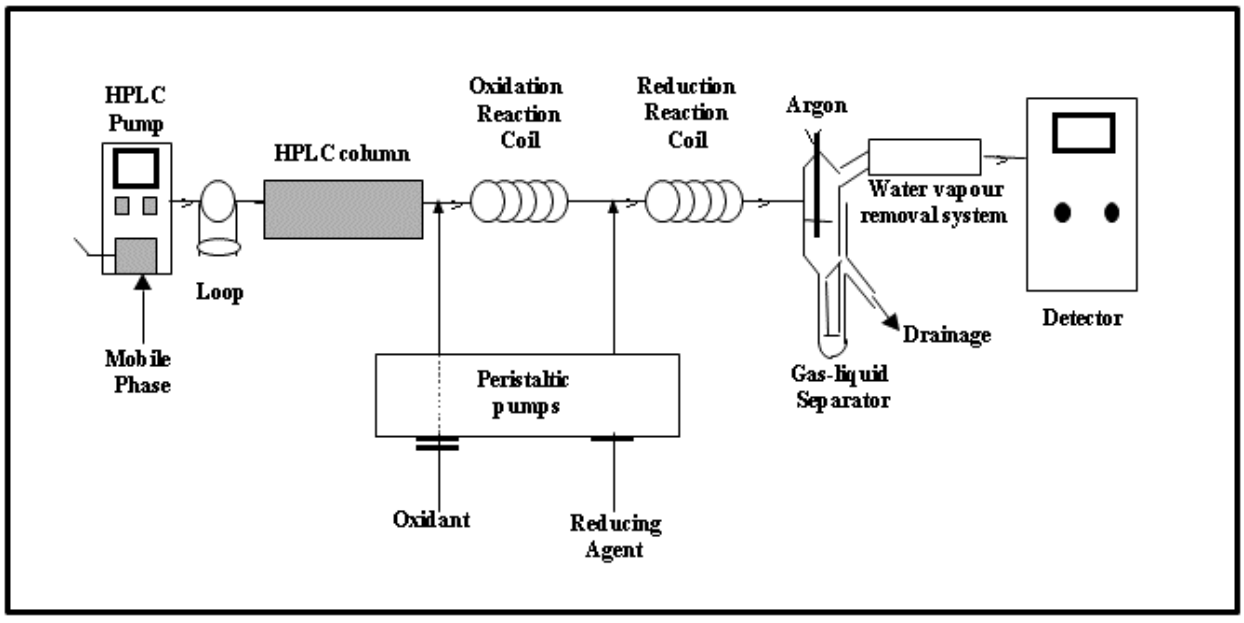


Figure 3. Schematic diagram of HPLC used for mercury compounds speciation.

Table 2. Solid phase extraction methods for mercury species preconcentration.

Complexing agent	Solid sorbent	Eluent	References
KBr	C18	$CH_3CN/H_2O/KBr$[1]	[115]
-	Develosil-ODS	Cysteine/acetic acid	[116]
APDC[2]	LiChroCART RP-C_{18}	APDC[2] in CH_3CN/H_2O (pH 5.5)	[117]
SPDC[3] (pH 6)	Hypersil ODS RP-C_{18}	SPDC[3] in CH_3CN/H_2O (pH 6.5)	[87, 118]
-	Zorbax ODS RP-C_{18}	Dithiocarbamate in MeOH/ CH_3CN/H_2O	[119]
-	HPIC-CG-5 ion exchange column	$CH_3COOH/NaClO_4$/ cysteine (pH 4.4)	[120]
-	Sep-pack C_{18} modified with 2-mercaptoethanol[4,5]	Acetonitrile[6]	[121, 122]
SPDC[3] (pH 5-5.5)	Hypersil ODS RP-C_{18}	SPDC[3] in CH_3CN/H_2O (pH=5.5-6)	[36, 37]
APDC[2]	RP-C18	$MeOH/CH_3CN/H_2O$	[61]
Methylthioglycolate (pH 7.0)	Nucleosil RP-18	Methylthioglycolate in MeOH/citric acid (pH 5.8)	[123]
PDC[7]	C18	MeOH	[124]
PDC[7]	Cigarette filter sorbent		[125]
Dithizone[4,5]	RP-C18		[126]
DDTP[8]	RP-C18	EtOH	[127]
APDC[2]	RP-C18	$MeOH/CH_3CN/H_2O$	[128]

[1]Potassium bromide is believed to counteract the possible ionic characteristic of mercury analyte; [2]Ammonium pyrrolidine dithiocarbamate; [3]Sodium pyrrolidine dithiocarbamate; [4]Off-line; [5]Used for in situ mercury concentration during water sampling because the adsorbed complexes on the cartridge are stable for at least 2 weeks; [6]Acetonitrile phase is rotary evaporated and the residue is dissolved in the mobile phase; [7]Pyrrolidinedithiocarbamate; [8]Dithiophosphoric acid diacyl ester.

after preconcentration on dithizone-anchored poly (ethylene glycol dimethacrylate-hydroxyethyl-methacrylate) microbeads, when the desorption medium was a mixture of copper sulphate and acidic potassium bromide for organomercury species or dilute nitric acid for inorganic mercury. Although the possibility of direct determination of mercury compounds adsorbed on solid sorbent by slurry sampling (SS) has also been investigated, it is not discussed in this work.

b) Mercury compounds separation

In reversed phase techniques, the mobile phase is a mixture of a polar organic solvent and water, containing a buffer or salts that control the analyte ionization. The most widely used organic solvents in the preparation of the mobile phase are methanol or acetonitrile, while the buffer preferred is ammonium acetate/acetic acid. However, complexing agents are also added to the mobile phase, which form stable complexes with organic and inorganic mercury compounds providing adequate sensitivity and selectivity [21]. The most used mobile and stationary phases for mercury compounds separation are shown in Table 3.

The suitability of N,N-disubstituted dithio-carbamates for separations on reversed-phases is due to the fast formation of strong and neutral

Table 3. Mobile and stationary phases for chromatographic separation of mercury species.

Mobile phase	Stationary phase	References
0.04 mol dm^{-3} cysteine in 0.1 mol dm^{-3} acetic acid (pH 2.9)	STR-ODS-H 5 µm packing	[116]
0.04 mol dm^{-3} cysteine in 0.1 mol dm^{-3} acetic acid (pH 2.9)	Bishoff ODS-H 5 µm packing	[48]
25 µmol SPDC[1] in CH_3CN/H_2O (pH 3.5)	Hypersil ODS 5 µm packing	[49]
0.5 mmol dm^{-3} SPDC[1] in CH_3CN/H_2O (pH 5.5-6.5)	Chemosphere ODS 5 µm packing	[131]
0.5 mmol dm^{-3} SPDC[1] in CH_3CN/H_2O (pH 5.5-6.5)	Hypersil ODS 3 µm packing	[55, 87]
0.5 mmol dm^{-3} APDC[2] in CH_3CN/H_2O (pH 5.5)	LiChrospher 5 µm packing	[117]
3 mmol dm^{-3} dithiocarbamate in $CH_3OH/CH_3CN/H_2O$	Zorbax ODS 5 µm packing	[119]
CH_3CN /1.5 mol dm^{-3} HCl	Hypersil ODS 5 µm packing	[132]
CH_3CN/0.005% 2-mercaptoethanol/0.2 mmol dm^{-3} DDAB[3] (pH 5)	Spherisorb ODS 2 10 µm packing bonded silica modified by passing 1 mmol dm^{-3} DDAB[3]	[121, 122, 133]
1.0 mmol dm^{-3} acetic acid/1.0 mmol dm^{-3} $NaClO_4$/5.0 mmol dm^{-3} cysteine (pH 4.4)	HPIC-CS-5 cation exchange separation column (Dionex)	[120]
CH_3OH/0.01 mol dm^{-3} TBABr[4] /0.025 mol dm^{-3} NaCl	Novapak 5 µm packing	[134]
CH_3OH/0.1 mmol dm^{-3} 2-mercaptoethanol/20 mmol dm^{-3} ammonium acetate	Chromspher 5 µm packing	[8]
1 mmol dm^{-3} SPDC[1] in CH_3CN /H_2O (pH=5.5-6)	Hypersil ODS	[36, 37]
0.1 mmol dm^{-3} 2-mercaptoethanol/20 mmol dm^{-3} ammonium acetate (pH 5) in CH_3OH/H_2O	Chromspher 3 µm packing	[29, 82]
CH_3OH/0.05% 2-mercaptoethanol (pH = 5)	Hypersil BDS	[76]
0.5% cysteine	Spherisorb ODS 2 packing bonded silica	[65]
5 mmol dm^{-3} ammonium pentanesulfonate as ion-pairing reagent in CH_3CN/H_2O		[135]
0.1% 2-mercaptoethanol/0.06 mol dm^{-3} ammonium acetate in $CH_3OH/CH_3CN/H_2O$	Vydac 201 TP 10 µm packing	[50]
0.5% cysteine as the ion pairing reagent (pH 5)	Spherisorb ODS 2 5 µm packing	[136]
10 mmol dm^{-3} TBABr[4], 0.01 % mercaptoethanol in CH_3OH/H_2O	Spherisorb ODS 2 5 µm packing	[137]
0.01% mercaptoethanol in CH_3OH/H_2O	Kromasil 5 µm packing	[92]
0.1 mmol dm^{-3} EDTA in CH_3OH/H_2O	Novapak	[138]
0.02 mmol dm^{-3} DDTC[5] in CH_3OH/H_2O	Zorbax 5 µm packing	[11]
0.1 mmol dm^{-3} mercaptobenzothiazole in CH_3OH/H_2O (pH 6.2)	5 µm packing	[139]
0.02% methylthioglycolate in CH_3OH/0.1 mol dm^{-3} citric acid (pH 5.8)	Hypersil ODS 5 µm packing	[123]
dithizone	CLC-ODS	[140]

Table 3 continued..

0.2 mmol dm^{-3} cetyltrimethylammonium hydrogensulfate water micellar media (pH 2) in CH_3CN/H_2O	Separon SGX 5 µm packing	[141]
20 mmol dm^{-3} ammonium acetate in CH_3OH/H_2O	Chromspher 5 µm packing	[24]
APDC[2] in CH_3OH	Zorbax Eclipse XDB 1.8 µm packing	[124]
$CH_3OH/CH_3CN/H_2O$/200 mmol dm^{-3} acetic acid (pH 3.5)		[142]
0.01% APDC[2] in $CH_3OH/CH_3CN/H_2O$ (pH 3.5)		[143]
50 mmol dm^{-3} pyridine/0.5% L-cysteine/5% CH_3OH (pH 2)	Hamilton PRP-X200 polymer-based cation-exchange column	[144]
0.1% L-cysteine/HCl/H_2O		[75]
0.06 mol dm^{-3} ammonium acetate/20 µg dm^{-3} Bi/0.1% 2-mercaptoethanol in CH_3OH/H_2O		[73]
0.06 mol dm^{-3} amonium acetate/0.1% cysteine in CH_3OH/H_2O (pH 6.8)	SphereClone ODS2 80A PEEK 5 µm packing	[67]
0.08% ammonium acetate/0.02% L-cysteine		[68]
micellar mobile phase containing 0.05 mol dm^{-3} CTMABr[6]/1% 2-propanol/0.001 mol dm^{-3} DCTA[7]/H_2SO_4 (pH 2)		[145]

[1]Sodium pyrrolidinedithiocarbamate; [2]Ammonium pyrrolidine dithiocarbamate; [3]Dodecyldimethylammonium bromide; [4]Tetrabutylammonium bromide; [5]Diethyldithiocarbamate; [6]Cetyltrimethylammonium bromide; [7]Cyclohexylenediaminetetraacetic acid.

complexes. Falter and Schöler [118] tested the use of sodium pyrrolidinedithiocarbamate (SPDC), sodium diethyldithiocarbamate (SDDC) and hexamethyleneammonium-hexamethylene-dithiocarbamate (HMA-HMDC) as complexing and preconcentration agents combined with different eluents. The best results corresponded to SPDC because the chromatograms obtained using SDDC and HMA-HMDC complexes show poorer results. In one case, the complexes were not quantitatively destroyed by ultraviolet irradiation and, in the other case the interaction with the column material was not suitable for a sufficient separation.

However, some authors reported that derivatization with diethylamine dithiocarbamate in presence of disodium ethylenediamine-tetraacetate is unsuitable for the determination of phenylmercury compounds by HPLC due to a side reaction in which diphenylmercury and the mercuric chelate were formed. Mercuric chelate formation is equivalent to half of the loss of phenylmercury complex [146].

Strategies based upon the introduction of vesicles into the chromatographic mobile phases can provide a greater variety of interactions with the analytes. Moreover, the richness of possibilities in such interactions could be manipulated in order to achieve the desired separation. In this sense, vesicle-mediated HPLC separation coupled to atomic spectroscopy detection provides a competitive, low-cost, efficient and robust separation which, at the same time, can substantially enhance the performance of atomic spectroscopic detectors, especially plasma detectors [147].

Ion-exchange chromatography requires a mobile phase containing a compound that forms complexes charged with mercury species, like cysteine gives compounds positively charged at low pH values [48, 65, 68, 75, 116, 120, 136, 144]. Ion-pair HPLC with tetra-n-alkylammonium bromides is effective for the separation of mercury species [134, 137, 148] because quaternary ammonium halide salts are effective extractants for mercury compounds, both inorganic mercury

and organomercury forming extractable anionic complexes in the presence of halide ions.

The most widely used chromatographic columns are packed with octadecylsilane (C_{18}), such as Hypersil ODS, Kromasil C18, LiChrospher RP-18, Spherisorb ODS-2, Novapak, Zorbax ODS, Chromspher RP18 and Chemosphere ODS. The Spherisorb ODS-2 columns can be purchased of particle size equal to 3, 5 or 10 µm and low pore size of 80 Å. The Hypersil ODS columns are well established and referenced in many HPLC methods as an excellent C18 phase for a broad range of applications. This material is endcapped and is available in 3, 5 and 10 µm particle sizes with a pore size of 120 Å. Hypersil BDS C18 receives a special base deactivation treatment during silica material manufacture to reduce silanophilic activity. It is endcapped and is available in 3 and 5 µm particle sizes with a high pore size (130 Å). Kromasil C_{18} consists of perfectly spherical and totally porous particles. The uniqueness of Kromasil high performance spherical silica is the combination of high surface area, mechanical strength, chemical purity, chemical stability, optimized surface properties and well-defined pore structure. Kromasil C18 and LiChrospher RP-18 present a particle size of 5 µm and a pore size of 100 Å. The Hypersil ODS columns have less surface area (170 m^2 g^{-1}), followed by Spherisorb ODS-2 (220 m^2 g^{-1}), Kromasil C18 (340 m^2 g^{-1}) and, finally, LiChrospher RP-18 (350 m^2 g^{-1}).

c) Oxidation of organomercury species

Inorganic mercury can be selectively determined using stannous chloride in acid medium as reducing agent due to the inability to reduce organomercury compounds [149, 150]. However, total mercury determinations are carried out using sodium borohydride due to its power to reduce both inorganic and organic mercury species when the sensitivity for the different mercury species does not differ significantly [150]. Although some workers report that sodium borohydride allows the determination of total mercury, other authors have obtained different sensitivity for methylmercury and inorganic mercury in several matrices when sodium borohydride is used as the reducing agent for total mercury determination [150-155]. The formation of methylmercury hydride (MeHgH) instead of elemental mercury may be the cause of the different behaviour of both species [18, 57, 156, 157]. This problem is solved by oxidation of organomercury compounds to inorganic mercury, previous to reduction to elemental mercury, using combinations of strong acids (hydrochloric, sulphuric and nitric acids), oxidants (hydrogen peroxide, potassium permanganate, potassium dichromate, potassium persulfate, potassium bromide-potassium bromate), high temperatures, ultraviolet irradiation, microwave exposure and sonolysis [151, 158, 159]. Stannous chloride can also be used for total mercury determinations involving a previous oxidative treatment step.

Post-column oxidation (PCO) requires to first mix chromatographic effluent with the oxidizing agent and to introduce this mixture into an oxidation coil in order to convert organic mercury compounds into mercury (II) ions. The stream is then mixed with the reducing agent and led into the reduction coil, where the reduction reaction takes place. Thus, the PCO allows quantitative destruction of mercury complexes, formed during the chromatographic separation, and conversion efficiency of methylmercury to inorganic mercury. The most used oxidants for on-line organomercury oxidation are shown in Table 4. Some of them are potassium persulphate in acid medium and potassium dichromate, which can be catalyzed by metallic ions like Cd (II) or Cu (II) [8, 25, 29, 48, 116]. Furthermore, microwave irradiation is also used for accelerating the oxidation reaction inside the oxidation coil [65, 163]. However, the use of ultraviolet irradiation for organomercury compounds oxidation presents one important advantage because it permits to avoid a potential contamination source as consequence of the absence of oxidizing reagents [36, 37, 43, 44, 55, 64, 74, 118, 131, 137].

d) Reduction to elemental mercury

Post-column mercury cold vapour generation (PCCVG) is an attractive approach because the possibility of molecular rearrangements during the derivatization reaction is avoided, providing that there is no post-column mixing of the separated analytes. The methodology is based on the generation of mercury vapour, from mercury species present in chromatographic effluent, into the chemifold where it is mixed with the reducing agent along a reduction coil. Then, mercury vapour is purged from the liquid phase with an argon stream in the gas-liquid separator and swept into a quartz T-cell (atomization cell) interposed

Table 4. Oxidizing and reducing reagents used for mercury determination in chromatographic effluents.

Oxidation	Reduction	Detection	References
2% $K_2S_2O_8$ + 65 mg dm^{-3} $CuSO_4$	2% $SnCl_2$ in 3.4 mol dm^{-3} NaOH	AAS	[116]
0.5% $K_2Cr_2O_7$ in 20% HNO_3	0.5% $NaBH_4$ in 0.05 mol dm^{-3} NaOH	AAS	[115]
1% $K_2Cr_2O_7$ + 100 mg dm^{-3} Cd^{2+}	0.5% $NaBH_4$ in 0.25% NaOH	AAS	[48]
2% $K_2S_2O_8$ + 0.25 mol dm^{-3} H_2SO_4 + 0.008 mol dm^{-3} $CuSO_4$	1.5 % $SnCl_2$ + 1.2 mol dm^{-3} NaOH	AFS	[25]
ultraviolet irradiation	1% $NaBH_4$ in 0.5 mol dm^{-3} NaOH	AAS	[131]
ultraviolet irradiation + 10% H_2O_2	3% $NaBH_4$	AAS	[132]
	1% $NaBH_4$ in 0.1% NaOH + 1% HCl	AAS	[121, 122]
0.25 mol dm^{-3} H_2SO_4 + 0.008 mol dm^{-3} $CuSO_4$ + 2 % $K_2S_2O_8$	1.5 % $SnCl_2$ + 1.2 mol dm^{-3} NaOH	AFS	[8]
	0.1% $NaBH_4$ in 0.1 mol dm^{-3} NaOH (pH =11.5)	AAS	[117, 120]
ultraviolet irradiation	1% $NaBH_4$ in 1 mol dm^{-3} NaOH (pH=13)	AAS	[118]
	0.1 mol dm^{-3} Cr(II)[1]	AFS	[160]
0.25 mol dm^{-3} H_2SO_4 + 0.008 mol dm^{-3} $CuSO_4$ + 2 % $K_2S_2O_8$	1.5 % $SnCl_2$ in 1.2 mol dm^{-3} NaOH	AFS	[29]
5 % $K_2S_2O_8$ in 0.5 mol dm^{-3} H_2SO_4+ 1.6 mol dm^{-3} $CuSO_4$	3 % $SnCl_2$ in 2.4 mol dm^{-3} NaOH		
ultraviolet irradiation	1% $NaBH_4$ in 0.5 mol dm^{-3} NaOH	AAS	[55]
ultraviolet irradiation	1 %$NaBH_4$ in 0.5 mol dm^{-3} KOH	AFS	[36, 37]
	0.1% $NaBH_4$ in 0.02 mol dm^{-3} NaOH	CV^2-PN^3-ICP-MS	[136]
ultraviolet irradiation	1% $NaBH_4$ in 0.5 mol dm^{-3} NaOH	CV^2-ICP-MS	[137]
	0.264 mol dm^{-3} $NaBH_4$ in0.125 mol dm^{-3} NaOH + thermolysis	AAS	[61]
	0.01% $NaBH_4$ in 1% NaOH	AAS	[150]
ultraviolet irradiation	1.5 % $SnCl_2$ in 1.2 mol dm^{-3} HCl	AFS	[43, 44, 64]
MW^4+ 0.18 mol dm^{-3} $K_2S_2O_8$ + 1.6mmol dm^{-3} $CuSO_4$	0.9 mol dm^{-3} $SnCl_2$ in 0.1 mol dm^{-3} HCl	AES	[65]
MW^4+ $K_2S_2O_8$ in HCl medium	(not refered)	AFS	[161]
$K_2S_2O_8$ in HCl	(not refered)	AFS	[142]
3% m/v $K_2S_2O_8$ in 10% HCl	(not refered)	AFS	[143]
ultraviolet irradiation	(not refered)	AFS	[74]

[1]Electrolytically generated; [2]Cold vapour; [3]Pneumatic nebulization; [4]Microwave.

in the optical beam of the spectrometer. The most widely used reducing agents are sodium borohydride in alkaline medium and stannous chloride in acid or alkaline medium, as above mentioned and can be seen in Table 4.

Yin *et al.* [162] reports a method for direct mercury vapour generation on nano TiO_2, under ultraviolet irradiation in the presence of a formic acid and sodium formate mixture, as a hole scavenger. A novelty designed UV/TiO_2 photo-catalysis reaction device (UV/TiO_2 PCRD) is used as an effective sample introduction unit and an interface for mercury species determination by atomic fluorescence spectrometry (AFS) and mercury speciation by HPLC-AFS. UV/TiO_2 PCRD is a superior alternative for online mercury vapour generation in comparison with the traditional potassium borohydride/sodium hydroxide-hydro-chloric acid system.

e) Detection techniques

After HPLC separation, the most used detection techniques for mercury compounds identification and quantification are the following:

- Ultraviolet-visible spectrophotometry [11, 20, 24, 82, 123, 139-141, 145, 148, 165, 167];
- Amperometry/Coulometry [168];
- Cold vapour-atomic absorption spectrometry (CV-AAS) [48, 61, 87, 115, 116, 118-122, 131, 132, 150, 164, 169];
- Cold vapour-atomic fluorescence spectrometry (CV-AFS) [25, 36, 37, 43, 74, 76, 143, 160, 162, 170, 173];
- Atomic emission spectrometry (AES) [65];
- Cold vapour-microwave induced plasma-atomic emission spectrometry (CV-MIP-AES) [133];
- Mass spectrometry (MS) [124,171];
- Inductively coupled plasma-mass spectrometry (ICP-MS) [50, 67, 73, 135, 144, 166, 172, 174];
- Cold vapour-inductively coupled plasma-mass spectrometry (CV-ICP-MS) [136].

2.3. Detection limit and linear range

Two important figures of merit that must always be considered when choosing an analytical technique are the detection limit and the linear range of the proposed method. Therefore, the detection limits and linear ranges found in GC and HPLC works are shown in Tables 5 and 6, respectively.

2.4. Advantages and disadvantages of the chromatographic separation methods

GC methods permit to detect lower concentrations than HPLC methods, taking into account the data shown in Tables 5 and 6. In fact, the lowest detection limit found is 0.12 pg of Hg [102], using GC as separation technique and ICP-MS as detection technique. The interfacing with element-specific detectors is also less complicated for GC than HPLC [97]. However, it should be stated that when using HPLC, the sample preparation is simpler [95] because it is not necessary to form volatile derivatives [8, 51, 61, 112] and, therefore, a smaller number of the sample preparation steps is involved [8, 61, 137, 175]. In HPLC the mercury compounds separation is performed at ambient temperature [82, 93, 96], eliminating thermal degradations [93]. Moreover, it is not required to carry out the column passivation and it is possible to determine low volatile or non volatile species, such as mersalylic acid [82, 93]. Using the HPLC technique, it is also possible to determine ethyl-mercury, which is not the case when ethylation is used as derivatization technique in GC, where inorganic mercury is derivatized to ethylmercury.

2.5. The most used detection techniques: a brief discussion

Several detectors have been coupled to HPLC and GC techniques, such as ECD, AAS, AFS, ICP-MS, ICP-AES, MIP-AES, MIP-PED, GD-AES, ACP-AES, spectrophotometry and amperometry/ coulometry. Nevertheless, direct coupling of liquid chromatography with detection technique can be technically complex and usually leads to sensitivity losses. Therefore, the investigation orientated to the design of new interfaces is growing. In fact, post-column derivatization of analytes results in better detection limits.

The ECD detector is non specific, being subjected to interferences caused by compounds that can be coextracted with organic mercury [93, 100], namely halogenated compounds [12]. Generally, when ECD is used, the reagents and the solvents used in the extraction must be previously cleaned or selected in order to prevent the presence of compounds that capture electrons [12]. This detector is very sensitive for compounds that contain at least one electronegative element as, for example, methylmercury chloride, being not

Table 5. Detection limits (µg dm^{-3} or ng) and linear ranges (µg dm^{-3} or ng) reported in GC works.

Reference	Detection	Mercury species	Detection Limit		Relative standard deviation (%)	Linear Range	
			(µg dm^{-3})	(ng)		(µg dm^{-3})	(ng)
[45]	AAS	MeHg	100(1)	167x10^{-3} (1)		Until 20x10^{3}	Until 33
[47]	AAS	Hg(II) MeHg		75x10^{-3} (1) 4x10^{-3} (1)			0.05-4.0 0.05-0.5
[7]	AFS	MeHg	0.015x10^{-3} (1) 0.050x10^{-3} (1)				
[27]	AFS	MeHg EtHg Me_2Hg	0.04(1) 0.04(1) 0.06(1)	0.2x10^{-3} (1) 0.2x10^{-3} (1) 0.3x10^{-3} (1)	(0.632 μg dm^{-3}; n=3) 1.5 (0.632 μg dm^{-3}; n=3) 1.5	Until 0.8 Until 0.8	
[80]	*AAS*	Hg(II) MeHg Me_2Hg Et_2Hg		0.11(1) 0.05(1) 0.05(1) 0.05(1)	(1 ng; n=3-5) 3-7% (1 ng; n=3-5) 3-7% (1 ng; n=3-5) 3-7% (1 ng; n=3-5) 3-7%		0.05-5 0.05-5 0.05-5 0.05-5
[106]	AFS	Hg(II) MeHg		1x10^{-3} (1) 2x10^{-3} (1)	(50 ng dm^{-3}; n=7) 8 (50 ng dm^{-3}; n=7) 8	Until 5x10^{-3} Until 20x10^{-3}	
[100]	AES	MeHg	1 - 0.8(1)	1.5x10^{-3}-1.2x10^{-3} (1)	(30 ng dm^{-3}; n=11) 4.4		
[28]	MIP-PED	MeHg Et_2Hg Me_2Hg	75 50 60	15x10^{-3} 10x10^{-3} 12x10^{-3}		375-75x10^{3} 200-60x10^{3} 350-90x10^{3}	0.075-15 0.04-12 0.07-18
[9]	AFS	MeHg EtHg	0.02x10^{-3} (1,2) 0.02x10^{-3} (1,2)	0.2x10^{-3} (1,2) 0.2x10^{-3} (1,2)	(0.4 μg dm^{-3}; n=3) 1.5 (0.4 μg dm^{-3}; n=3) 1.5	Until 4 Until 4	
[102]	ICP-MS	MeHg Et_2Hg PhHg Hg(II)		120x10^{-6} (1)	(20 pg; n=6) 4.6(3)/5.8(4) (20 pg; n=6) 2.2(3)/4.2(4)		1x10^{-3}-1 1x10^{-3}-1 1x10^{-3}-1
[12]	AFS	MeHg EtHg		0.2x10^{-3} (1,2) 0.2x10^{-3} (1,2)			
[13]	AAS	Hg(II) MeHg	4x10^{-4} (1,5) 2.2x10^{-3} (1,6) 3x10^{-5} (1,5) 1.7x10^{-4} (1,6)	5x10^{-4}(5)-2.8x10^{-3}(1,6) 5x10^{-4}(5)-2.8x10^{-3}(1,6)			(0.5-35)x10^{-3}(5) 2.8x10^{-3}-1.6(6) (0.5-35)x10^{-3}(5) 2.8x10^{-3}-1.6(6)
[56]	ECD	MeHg (packed column) MeHg (capillary column)		25x10^{-3} (1) 2x10^{-3} (1)	(100 μg dm^{-3}; n=10) 0.95 (100 μg dm^{-3}; n=10) 0.43		25x10^{-3}-1 2x10^{-3}-0.2

Table 5 continued..

[35]	AAS	Hg(II) MeHg	$1x10^{-3}$ $1x10^{-3}$	$50x10^{-3}$ $50x10^{-3}$	(0.1 μg dm^{-3}; n=3) <10 (0.1 μg dm^{-3}; n=3) <10	0.01-0.4 0.01-0.4	0.5-20 0.5-20
[57]	AAS	Hg(II) MeHg	$1x10^{-3}$ $1x10^{-3}$	$50x10^{-3}$ $50x10^{-3}$	(5 ng; n=10) <10 (5 ng; n=10) <10	0.01-0.4 0.01-0.4	0.5-20 0.5-20
[17]	MIP-AES	Hg(II) MeHg	$2x10^{-3}$ (1) $5x10^{-5}$ (1)	$2.0x10^{-3}$ (1) $5.0x10^{-4}$ (1)	(0.2 ng) 5 (0.2 ng) 5		0.1-10 0.05-10
[18]	AAS	Hg(II) MeHg		$50x10^{-3}$ (1) $50x10^{-3}$ (1)	(1 ng) 10 (1 ng) 10		0.5-50 0.5-50
[58]	GD-AES	MeHg EtHg Hg(II)	1.3(1) 1.3(1) 3.0(1)	$1.3x10^{-3}$ (1) $1.3x10^{-3}$ (1) $3.0x10^{-3}$ (1)	(100 μg dm^{-3}; n=10) 4.2 (100 μg dm^{-3}; n=10) 5.0 (100 μg dm^{-3}; n=10) 7.0	Until $2.5x10^{3}$ Until $2.5x10^{3}$ Until $2.5x10^{3}$	
[40]	ICP-MS	MeHg	$15x10^{-3}$ (7)	$0.15x10^{-3}$ (7)			
[62]	AFS	MeHg	0.025(1)	$0.25x10^{-3}$ (1)	($86x10^{3}$ ng dm^{-3}; n=10) 7		
[62]	ICP-MS	MeHg	0.9(1)	0.9(1)	($1x10^{3}$ ng dm^{-3}; n=5) 9	Until 180	Until 180 pg
[104]	MIP-AES	Hg(II) MeHg Me_2Hg	 $0.8x10^{-3}$ (1) $0.4x10^{-3}$ (1)	 $3.2x10^{-3}$ (1) $1.6x10^{-3}$ (1)	($1x10^{3}$ ng dm^{-3}; n=5) 5 ($1x10^{3}$ ng dm^{-3}; n=5) 5 ($1x10^{3}$ ng dm^{-3}; n=5) 13		
[63]	AES	MeHg	0.04	$0.04 x10^{-3}$ (1)	($18x10^{3}$ ng dm^{-3} - Derivatised standard solution; n=8) 5.5 (Derivatised biological sample; n=5) 3.5		
[66]	MIP-AES	Hg(II) MeHg	0.86(8) 0.12(8)		(TORT-1; n=5) 12.9 (TORT-1; n=5) 6.7	0.1-8.0 0.2-3.0	
[71]	MS	MeHg	10(1)	$10x10^{-3}$ (1)	(Seven replicates; Intra-day) 5.8 (Seven replicates; Inter-day) 7.6		
[19]	MS	Hg(II) MeHg EtHg	$1.0x10^{-3}$ (9) $1.5x10^{-3}$ (9) $1.5x10^{-3}$ (9)	$50x10^{-3}$ (8) $75x10^{-3}$ (8) $75x10^{-3}$ (8)	(50 ng dm^{-3}; n=11) 6.3 (50 ng dm^{-3}; n=11) 7.0 (50 ng dm^{-3}; n=11) 7.6	0.003-0.8 0.004-1.0 0.004-1.0	0.15-40 0.2-50 0.2-50

[1]The detection limit was determined as three times the baseline noise; [2]With pre-concentration; [3]In terms of area; [4]In terms of height; [5]Measurements performed at the wavelength of 184.9 nm; [6]Measurements performed at the wavelength of 253.7 nm; [7]The detection limit was determined as two times the baseline noise; [8]The detection limit was determined as three times the standard deviation estimated in the regression analysis divided by the slope of the calibration line; [9]The detection limit was determined as three times the standard deviation of the blank divided by the slope of each calibration graph.

Table 6. Detection limits (μg dm^{-3} or ng) and linear ranges (μg dm^{-3}) reported in HPLC works.

Reference	Detection	Mercury species	Detection Limit (μg dm^{-3})	Detection Limit (ng)	Relative standard deviation (%)	Linear Range (μg dm^{-3})
[163]	ACP-AES	MeHg EtHg		70(1) 70(1)	(140 ng; n=5) <10	
[116]	CV-AAS	Hg(II) MeHg	1.0(1,2) 1.0(1,2)	0.1(1,2) 0.1(1,2)	(40 μg dm^{-3}; n=4) 1.4	Until 50 Until 50
[115]	CV-AAS	Hg(II) MeHg EtHg	0.78(2) 0.78(2) 0.42(2)		(6 μg dm^{-3}; n=5) 5.2 (6 μg dm^{-3}; n=5) 6.1 (6 μg dm^{-3}; n=5) 2.8	
[82]	UV	MeHg Other species	14.6(3) 7.0-95.1(3)		(1x10^3 μg dm^{-3}; n=9) 3.7 (1x10^3 μg dm^{-3}; n=9) 4.2-19.1	14.6-10x10^3
[135]	DIN(4)-ICP-MS	Hg(II) MeHg EtHg PhHg		3.0x10^{-3} (1) 7.0x10^{-3} (1) 7.0x10^{-3} (1) 6.0x10^{-3} (1)	(2 ng; n=5) 2.7 (2 ng; n=5) 2.7 (2 ng; n=5) 2.8 (2 ng; n=5) 3.0	
[140]	UV	Hg(II) MeHg EtHg PhHg		0.10(1) 0.10(1) 0.30(1) 0.10(1)		
[24]	UV	MeHg		0.5		
[123]	UV	MeHg EtHg		25(1) 19(1)		
[139]	UV	Hg(II) MeHg EtHg PhHg		0.5(1) 0.3(1) 0.4(1) 0.4(1)	(5 ng; n=7) 3.1 (5 ng; n=7)2.1 (5 ng; n=7) 2.6 (5 ng; n=7) 3.4	
[120]	CV-AAS	Hg(II) MeHg EtHg PhHg	0.015(1,2) 0.50(1,2) 0.09(1,2) 0.50(1,2)			
[164]	CV-AAS	Hg(II) MeHg	4(3) 3(3)		8.2 6.3	
[48]	CV-AAS	Hg(II) MeHg EtHg		0.5(3) 0.8(3)	(200 μg dm^{-3}; n=6)<2 (200 μg dm^{-3}; n=6)<2	Until 200 Until 300

Table 6 continued..

[25]	CV-AFS	MeHg and EtHg, Methoxyethyl, ethoxyethyl and Hg benzoate Aromatic species that eluted latter	0.8(1) 0.8(1) 1.4(1)	20x10^{-3} (1)	(800x10^{3} μg dm^{-3}; n=5) <6	2-400
[50]	USN(5)-ICP-MS	Hg(II) MeHg EtHg	0.4 0.7 0.8	0.08(1) 0.14(1) 0.16(1)	(10 μg dm^{-3}; n=5) 3.1 (20 μg dm^{-3}; n=5) 4.2 (20 μg dm^{-3}; n=5) 4.9	
[121]	CV-AAS	Hg(II) MeHg Hg(II) MeHg	16(1) 10(1) 0.16(1,2) 0.1(1,2)		(20 ng; n=8) 6.9 (20 ng; n=8) 3.5	16-400 10-400
[131]	CV-AAS	Hg(II) MeHg EtHg PhHg	4.0(1) 4.0(1) 4.0(1) 4.0(1)	80x10^{-3} (1) 80x10^{-3} (1) 80x10^{-3} (1) 80x10^{-3} (1)	(0.25x10^{3} μg dm^{-3}) 5 (0.25x10^{3} μg dm^{-3}) 5 (0.25x10^{3} μg dm^{-3}) 5 (0.25x10^{3} μg dm^{-3}) 6	10-1000 10-1000 10-1000 10-1000
[122]	CV-AAS	MeHg	16(1) 10(1) 0.16(1,2) 0.1(1,2)			
[120]	CV-AAS	Hg(II) MeHg EtHg Hg(II) MeHg EtHg	10.0(1) 50.0(1) 20.0(1) 0.02(1,2) 0.10(1,2) 0.04(1,2)	 2(1,2) 10(1,2) 4(1,2)		
[132]	CV-AAS	Me_2Hg Et_2Hg	30(1) 30(1)			
[148]	UV	Hg(II) MeHg EtHg PhHg BzHg		0.8 8.0 3.8 1.3 0.2		
[118]	CV-AAS	Hg(II) MeHg EtHg MeOEtHg EtOEtHg PhHg	0.5 x10^{-3} (2) 0.5 x10^{-3} (2) 0.5 x10^{-3} (2) 0.5 x10^{-3} (2) 0.6 x10^{-3} (2) 0.6 x10^{-3} (2)		(5 ng) 7 (5 ng) 6 (5 ng) 6 (5 ng) 9 (5 ng) 9 (5 ng) 7	

Table 6 continued..

[165]	UV	Hg(II) MeHg	10[(1)] 25[(1)]			0.05x10^3- 100x10^3 0.1x10^3-50x10^3
[133]	CV-MIP-AES	Hg(II) MeHg	0.15 0.35		(20 μg dm^{-3}; n=5) 6.7 (50 μg dm^{-3}; n=5) 6.8	
[160]	CV-AFS	MeHg EtHg PhHg	5-7			0-100 ng 0-100 ng 0-100 ng
[11]	UV	Hg(II) MeHg EtHg PhHg		0.72 0.25 0.21 0.19	3-3.7	
[166]	ICP-MS	Hg(II) MeHg EtHg	0.5[(3)] 0.5[(3)] 1.0[(3)]		(1.0 μg dm^{-3}; n=3) 2.5 (1.0 μg dm^{-3}; n=3) 3.8 (1.0 μg dm^{-3}; n=3) 6.1	0 - 50 0 – 50 0 - 50
	ICP-MS	Hg(II) standards in seawater MeHg standards in seawater EtHg standards in seawater	0.25[(3)] 0.25[(3)] 0.75[(3)]		(1.0 μg dm^{-3}; n=3) 3.8 (1.0 μg dm^{-3}; n=3) 4.5 (1.0 μg dm^{-3}; n=3) 5.5	0 - 50 0 – 50 0 - 50
[167]	Visible	Hg(II) MeHg PhHg	41 78 220	1.15 2.18 6.16		0.04x10^3-0.4x10^3 0.1x10^3-1x10^3 0.6x10^3-3x10^3
[141]	Visible	Hg(II) MeHg PhHg	14 20 73	1.0 1.4 5.1		
[119]	CV-AAS	Hg(II) MeHg EtHg PhHg	1.92x10^{-3} [(2)] 0.86x10^{-3} [(2)] 1.94x10^{-3} [(2)] 1.06x10^{-3} [(2)]		3.8-7	
[87]	CV-AAS	MeHg		40x10^{-3} [(1,2)]		

Table 6 continued..

[36, 37]	CV-AFS	Hg(II) MeHg EtHg PhHg Mersalyl acid	$2x10^{-3}$ (1,2) $1.6x10^{-3}$ (1,2) $2x10^{-3}$ (1,2) $2x10^{-3}$ (1,2) $1.6x10^{-3}$ (1,2)	$8.0x10^{-3}$ (1,2)	(200 pg) 7 (200 pg) 4 (200 pg) 4 (200 pg) 7 (200 pg) 4	(20pg – 1000pg) (20pg – 1000pg) (20pg – 1000pg) (20pg – 1000pg) (20pg – 1000pg)
[168]	Amperometric/ Coulometric	Hg(II) MeHg EtHg PhHg	10-80(1) 8-50(1) 9-70(1) 20-140(1)	0.20-1.6(1) 0.16-1.0(1) 0.18-1.4(1) 0.40-2.8(1)	For all species: (2.0 μg cm^{-3}) 1.3-2.8(6) (0.3 μg cm^{-3}) 0.6-1.9(7)	
[136]	CV-PN(8)-ICP-MS	Hg(II) MeHg EtHg	0.11(1) 0.03(1) 0.04(1)	$3.0x10^{-3}$ (1)	(5 μg dm^{-3}; n=5) 1.6 (5 μg dm^{-3}; n=5) 1.5 (5 μg dm^{-3}; n=5) 3.3	
[61]	CV-AAS	Hg(II) MeHg EtHg PhHg	0.005(1,2) 0.009(1,2) 0.006(1,2) 0.010(1,2)		(0.5 μg dm^{-3}; n=9) 7.6 (0.5 μg dm^{-3}; n=9) 3.6 (0.5 μg dm^{-3}; n=9) 5.5 (0.5 μg dm^{-3}; n=9) 10.4	0.008-5 0.009-5 0.006-5 0.010-5
[145]	Visible	Hg(II) MeHg PhHg		1-3 1-3		
[150]	CV-AAS	Hg(II) MeHg	11.3(1) 13.2(1)	1.13(1) 1.32(1)	(500 μg dm^{-3}; n=10) 5.55 (500 μg dm^{-3}; n=10) 4.28	
[169]	CV-AAS	Hg(II) MeHg	$3.4x10^{-3}$ $1.7x10^{-3}$			
[43]	CV-AFS	Hg(II) MeHg	$51x10^{-3}$ (1)	10(1) 10(1)		
[65]	AES	Hg(II) MeHg	1.2(1) 1.8(1)		(100 μg dm^{-3}; n=3) 5 (100 μg dm^{-3}; n=3) 8	<1000 <1000
[67]	ICP-MS	Hg(II) MeHg	0.5 0.5			0.5-20
[170]	CV-AFS	Hg(II) MeHg	11 8			11-500 8-500
[171]	ESI(9)-MS	MeHg	39			
[73]	ICP-MS	Hg(II) MeHg	0.3 0.2			
[172]	ICP-MS	Hg(II) MeHg	0.9 1.1			

Table 6 continued..

[76]	CV-AFS	Hg(II) MeHg EtHg PhHg	0.07 0.2 0.12 0.06		(5 μg dm^{-3}; n=10) 5.3 (5 μg dm^{-3}; n=10) 3.0 (5 μg dm^{-3}; n=10) 4.4 (5 μg dm^{-3}; n=10) 3.4	
[143]	CV-AFS	Hg(II) MeHg EtHg PhHg	0.19-0.27 0.19-0.27		(1 μg dm^{-3}; n=5) 1.8-2.8	
[173]	CV-AFS	Hg(II) MeHg EtHg	4 3 2		(n=7) 2.9 (n=7) 2.0 (n=7) 2.4	10-1000 10-1000 10-1000
[74]	CV-AFS	MeHg EtHg	0.6 1			
[20]	UV	MeHg EtHg PhHg	3.8 0.7 0.3		(30 μg dm^{-3}; n=7) 8.9 (30 μg dm^{-3}; n=7) 6.4 (30 μg dm^{-3}; n=7) 6.6	5-100 1-100 1-100
[174]	ICP-MS	Hg(II) MeHg	2.5 2.0			
[124]	API$^{(10)}$-MS	Hg(II) MeHg EtHg PhHg	0.090 0.370 0.280 0.250			
[162]	CV-AFS	MeHg EtHg PhHg	0.81 0.20 0.87			
[144]	ICP-MS	Hg(II) MeHg	0.05 0.08	1.0×10^{-3} 1.6×10^{-3}		

[1]The detection limit was determined as three times the background noise; [2]With pre-concentration; [3]The detection limit was determined as two times the background noise; [4]Direct injection nebulizer; [5]Ultrasonic nebulizer; [6]Amperometric detection; [7]Coulometric detection; [8]Pneumatic nebulizer; [9]Electrospray ionization; [10]Atmospheric pressure ionization.

sensitive for dimethylmercury. Detection limits comprished between 2 and 25 pg are reported for the GC-ECD coupling [56].

Cai *et al.* [107] studied several hyphenated analytical techniques and concluded that the main advantage of GC-AES is its capability for multi-elemental analysis. Additionally, atomic emission is advantageous in the case of methyl elements, because their intense emission and low spectral background provide excellent sensitivity and a high degree of selectivity, as stated by Carro and Mejuto [95]. However, when real samples are analyzed by AES, strange peaks appear nearby the methylmercury peak, but methylmercury quantification is still possible [100]. AES (ICP-AES and MIP-AES) or MS (ICP-MS) have unique analytical capabilities for performing speciation studies, like excellent sensitivity and selectivity, as well as multi-elemental detection [62, 96, 176]. However, their high instrumental and running costs make them more difficult to be adopted widely as common chromatographic detectors. The detection limits for GC-AES vary between 0.04 and 3 pg for methylmercury, and between 2 and 3 pg for inorganic mercury.

The coupling between HPLC and AES is simple because this detector accepts continuous flows of HPLC effluent. The main disadvantage is the low tolerance of plasmas to organic solvents present in the mobile phases typically used in HPLC. Furthermore, the ineffectiveness of the nebulization system involves background noise, instability, a worsening of detection limits and, even, eventual extinction of the plasma. The problems previously mentioned can be solved by the use of alternative HPLC mobile phases which do not use organic solvents. In this sense, micellar liquid chromatography (MLC) presents several advantages [177] including enhanced selectivity, versatility, rapid gradient elution capability, low toxicity, low cost and the ability to simultaneously chromatograph both hydrophilic and hydrophobic solutes [178], but also shows some drawbacks including loss of efficiency and solvent strength [179].

There are three principal plasma sources that have been evaluated as specific detection modes in HPLC, namely direct current plasma (DCP), inductively coupled plasma (ICP) and microwave-induced plasma (MIP). The compatibility of DCP with a wide variety of solvents has facilitated its combination with HPLC. However, the interface of HPLC and MIP has been more challenging because of the low tolerance of the MIP towards organic solvents typically used in HPLC. The vesicular mobile phases are already being used for mercury compounds separation by HPLC with mercury vapour generation prior to MIP-AES detection in order to solve this incompatibility [133]. The detection limits are 0.15 and 0.35 μg dm^{-3} for inorganic mercury and methylmercury, respectively.

Colon and Barry [163] developed an alternating current plasma (ACP) detector for HPLC, using a glass-frit nebulizer as the interface that generates a very fine mist with droplet size distribution smaller than the pneumatic nebulizer and greatly enhances the introduction of organic solvents to the plasma. The potential of glow discharge (GD) device for the detection of the volatile covalent species of the analyte has also been explored recently, using cold vapour generation approach and applying radiofrequency (RF) powered GD device. The figures of merit obtained are very satisfactory, being its detection limits less than those reported for other common AES approaches like as ICP [65].

The MS detection is very useful for structural confirmation, being the analytes identified not only from their retention time, but also on the basis of distinctive features of their fingerprint mass spectra [19]. Bouyssiere *et al.* [113] also refer that the plasmas existent in ICP-MS systems are more robust and the detection limits are lower than the ones achieved with MIP-AES. Nevertheless, their high cost is the most important hindrance, making their acquisition for routine analysis very difficult [180]. Besides, the ICP-MS detectors present memory effects [136], being necessary lengthy washing periods [181, 182]. In fact, mercury seems to be able to adhere to the walls of the nebulization chamber, as well as to the tubes, causing sample contamination and sensibility loss [182]. This phenomenon can be due to the increase observed of mercury vapour pressure that is generated in the nebulization chamber, as a consequence of the increase of mercury volatility during pneumatic nebulization of high pressure [182]. Several solutions have been reported, namely the use of alternative

systems for sample introduction, such as direct injection nebulization (DIN) [182], which seems to be very promising. The detection limits reported for GC-ICP-MS coupling are in the range of 0.1 pg.

Other difficulties can be found when using the HPLC-ICP-MS systems, linked to the use of mobile phases rich in organic modifiers [96, 136, 137] or in salts [137]. A high quantity of organic modifier can induce plasma destabilization, signal enlargement and carbon deposition on the sampler and skimmer cones [137]. Furthermore, a high quantity of salts can provoke sampler obstruction [137]. An ICP-MS ability is the determination of the isotopic ratio. Thus, isotope dilution (ID) provides high accuracy quantification [77], being more precise double IDMS than single IDMS [183].

Conventional nebulizers introduce only 1-3% of the sample into the plasma and have large dead volumes, which can cause band broadening. In fact, the nebulizer is generally recognized to be one of the weakest components of the entire ICP-MS apparatus. The ultrasonic nebulizer (USN) has been satisfactorily coupled with HPLC-ICP-MS [50, 184]. On the other hand, the direct injection nebulizer (DIN) is a microconcentric pneumatic nebulizer placed inside the ICP torch. It has a low dead volume and produces a mist of fine droplets. The improvement in absolute detection limits (pg), in comparison with conventional nebulizers, is expected because all of the sample reaches the plasma with the DIN [135]. The low efficiency of the pneumatic nebulizers (PN) can also be overcome using other nebulizer types, like high performance flow/hydraulic high pressure nebulizing (HPF/HHPN) [37]. Moreover, the use of the cold mercury vapour generation technique increases mercury signal significantly. In this sense, a simple in situ nebulizer/vapour generation system is employed as a sample introduction device in HPLC-ICP-MS for mercury speciation determination [136]; thus, the entire injected sample is nebulized. The detection limit obtained is 3 pg. A cooled spray chamber is used to reduce the amount of organic vapour in the plasma and allow the use of large proportions of organic modifier in the eluent, without destabilizing the plasma. Carbon deposition from the modifier is reduced by the addition of oxygen to the plasma (post-nebulization) [77, 137].

A direct injection high efficiency nebulizer (DIHEN) interface for microbore HPLC-ICP-MS was also developed. This simple and relatively low-cost interface consists of a 1-piece micro-nebulizer, positioned in the ICP torch for the direct nebulization of solution into the base of the argon plasma [185]. The detection limits are in the low- to sub-pg range. It is important to take into account that no plasma instability or carbon deposition on the nebulizer tip was observed using organic modifiers in the mobile phase of up to 20%.

The main drawback of the previous HPLC-ICP-MS methods is that they do not provide structural information on mercury compounds and the identification relies solely on retention times, which could be confounded in the presence of a complex sample matrix or unidentified mercury containing compounds. Atmospheric pressure ionization mass spectrometry (API-MS) permits to determine the structure of mercury species eluting from a HPLC column and, therefore, to confirm their identity [92, 124].

The ability of particle beam/electron ionization-mass spectrometry (PB/EI-MS) to provide elemental and molecular information of a sample solution has been evaluated for the speciation of inorganic and organic mercury compounds. Specifically, the EI process yields mass spectra which reflect the chemical species eluting from the chromatographic column, either atom or molecules. Therefore, it is believed that the PB/EI-MS technique is well suited not only for mercury speciation, but also for obtaining comprehensive speciation information via atomic and molecular mass spectral information of diverse species and, thus, it can be used to solve speciation challenges [186]. The limits of detection are in the range of ng dm^{-3} (sub-ng).

CV-AAS is one of the most popular techniques, due to its simplicity, inexpensive instrumentation, speed, reliability, ready acceptance of liquid samples and availability. However, its sensitivity is not sufficient to perform speciation studies in some real samples with low mercury concentrations, such as waters. Besides, CV-AAS is subjected to spectral interferences proceeding from volatile organic compounds, such as acetone, toluene and benzene. The detection limits published for GC-AAS coupling are in the range of pg, while those reported for HPLC-CV-AAS systems are in the range of ng.

AFS is less susceptible to these kinds of interferences, being more sensitive than AAS [112]. The GC-AFS system has the advantage of simple operation and comparatively low cost [107]. The detection limits for this hyphenated system are in the range of 0.1 ng. Moreover, the HPLC-CV-AFS systems allow the injection of high sample volumes, are robust, imply easy operation and allow automation. The principal disadvantages are related to mercury fluorescence quenching. In fact, mercury fluorescence is suppressed by polyatomic molecules, namely, nitrogen, oxygen, hydrogen, organic eluents and, especially, water [25, 36, 37, 93, 187]. Photo-induced chemical vapour generation with formic acid, instead of the conventional potassium persulfate/sodium borohydride system, is a novel interface for HPLC-AFS hyphenated system. The new system is simple, environmentally benign, and inexpensive [162]. The detection limits for this coupling are in the range of ng.

In some speciation methods, a non-selective detection technique is used, such as ultraviolet-visible spectrophotometry [24, 82, 138]. Nevertheless, this is rarely applied to mercury speciation due to its low sensitivity and the lack of chromophores, which preclude the direct use of simple ultraviolet (UV)-visible detection. Thus, a preconcentration step and a derivatization procedure with organic complexing agents, prior to separation, are required. A series of new reagents is being investigated for the detection of their complexes by spectrophotometry. On the other hand, the problems associated with mercury speciation using HPLC as chromatographic separation technique are derived from the detector incompatibility with the continuous flow of mobile phase. The detection limits obtained are less than 25 ng.

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