Review article

Analytical strategies of sample preparation for the determination of mercury in food matrices — A review

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ABSTRACT

The present paper reports the advantages, drawbacks and applications of the main techniques of sample preparation employed during the determinations of total mercury and methylmercury in food matrices employing analytical methods such as: cold vapor atomic absorption spectrometry (CV AAS), cold vapor atomic fluorescence spectrometry (CV AFS), inductively coupled plasma mass spectrometry (ICP-MS), voltammetry, and neutron activation analysis. The use of slurry sampling, solid sampling, microwave assisted extraction, reflux system by cold finger and Vigreux column was discussed. Also a brief text on the use of chromatographic techniques for the speciation analysis of mercury is presented. A list of 134 references is cited and the analytical characteristics of some of these procedures proposed are shown as tables.

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1. Introduction

1.1. Human exposure to mercury and its compounds

Mercury and its organic and inorganic species are toxic to the central and peripheral nervous systems; however, this toxicity depends on its chemical form, among which organic mercury compounds are the most toxic. The principal chemical forms of mercury are: elemental mercury (Hg0), divalent inorganic mercury (Hg2+), methyl mercury (CH3Hg+) and dimethylmercury ((CH3)2Hg) [1]. The biomonitoring of mercury exposure depends on the chemical form. So, blood and urinary mercury are used to assess occupational exposure in case of contamination by elemental mercury. For exposure by methylmercury, the bioindicators used are blood and hair samples [1]. Mercury species are volatile and this is alarming because the inhalation of mercury vapor and its compounds produces harmful effects on the nervous, digestive and immune systems and the lungs and kidneys. Children and babies are most affected by mercury toxicity. Mercury releases in the environment originated from natural sources such as volcanoes and/or human activity as production of non-ferrous metal (smelters), cement, caustic soda and also waste disposal. In humans, the main contamination by mercury comes from foods, especially fish and seafood. In fishes, elemental mercury is naturally transformed into methylmercury, a compound of great toxicity [2–4]. Plant foods may also be contaminated by mercury due to the use of phosphate fertilizers in agricultural processing [5]. These inputs often contain appreciable amounts of toxic elements such as: mercury, cadmium, and lead. Jesus et al. determined mercury in six phosphate fertilizer samples [5]. The mercury content found varied from 34 to 210 μg kg−1. In this way, several plant foods can contain mercury as contaminant. Rice grown in contaminated regions accumulates mercury. It is of concern because mercury can be methylated by bacteria to produce methylmercury. So, many methods have been published for the determination of methylmercury in rice [6–9]. The World Health Organization (WHO) recommends a maximum intake of methylmercury of 1.6 μg kg−1 per week [10].

1.2. Analytical methods

The determination of mercury in food samples can be established in two steps: firstly, a sample preparation procedure is required and afterward the quantification step. The sample preparation step is complicated because of the volatile character of mercury and its species [11]. However, the mercury determination step is relatively simple and it has been often performed using the vapor generation technique coupled to atomic absorption spectrometry (CV AAS), fluorescence spectrometry (CV AFS), inductively coupled plasma optical emission spectrometry (CV ICP OES) and inductively coupled plasma mass spectrometry (CV ICP-MS) [12–16]. The vapor generation reaction can be performed employing NaBH4 or SnCl2 in acid medium as reducing agent [17–19]. NaBH4 promotes faster and more effective reduction so that it is preferred to SnCl2. However, the use of NaBH4 promotes the formation of an excess of H2 resulting in spectral interferences.

Some problems and interference are encountered in the determination of mercury species by ICP-MS [20]. For example, a significant memory effect is observed in the sample introduction system, resulting in long washing times and poor accuracy and reliability.

It is suggested that this problem is caused by the adherence of mercury to the walls of the spray chamber and the transfer tubing of the introduction system [21]. The mercury vapor builds up slowly in the spray chamber, due to the volatility of the element that increased by large pressure during the pneumatic nebulization. This effect has been eliminated by some different approaches, such as adding gold to the samples and standards as a stabilizing agent and using a combination of flow injection sample introduction [22]. Additionally, the procedures utilizing isotope dilution inductively coupled plasma mass spectrometry (IDA ICP-MS) have been proposed in recent years [23–26]. Clemens et al. published a review paper about speciation analysis of mercury in seafood using this technique. Several methods proposed were discussed [2].

Mercury has been determined also using electrothermal atomization atomic absorption spectrometry (ET AAS) [27,28]. Alternatively, voltammetric methods have been also proposed for the determination of mercury in several matrices. These are suitable for the determination of mercury in food samples because they are simple, fast, inexpensive and involve an instrumentation that can easily be operated. Further, the voltammetric methods have low limits of quantification because they are established as having a pre-concentration step of mercury on the working electrode and subsequent stripping. A review recently published summarizes the methods proposed for the determination of mercury employing electroanalytical techniques including applications for the analysis of food samples [29].

The neutron activation analysis (NAA) is a direct analysis technique employed for many matrices including foods, which have advantages such as: non-destructive character of the samples, good accuracy, ability to multi-elemental determinations and high selectivity and sensitivity. This is a good alternative for the determination of mercury considering the volatile nature of this element and the lower limits of quantification that can be obtained [30,31].

2. Determination of mercury in food matrices employing direct analysis

2.1. Direct Mercury Analyzer (DMA)

The use of the Direct Mercury Analyzer (DMA) is one of the good alternatives that have been proposed for the determination of trace amounts of mercury in complex matrices, including foods [32–40]. This equipment can be employed for the analyses of solid and liquid samples and it does not require any pre-treatment of these. It operates on a basis of sample thermal decomposition, followed by mercury catalytic reduction and amalgamation on a gold system for vapor mercury trapping and, subsequently mercury desorption and analytical technique measured by atomic absorption spectrometry. The efficiency of a chemical analysis using DMA depends strongly on the sample homogeneity which is being processed. Generally, the calibration curves are established using certified reference materials but, eventually can be performed also by external calibration technique employing aqueous standard solutions [36,39]. In this way, DMA has been used for the determination of mercury traces in several matrices [8,32,33]. Ipolyi et al. proposed a method for the determination of total mercury and methylmercury in mussel samples. Total mercury was quantified using DMA and methylmercury by gas chromatography–mass spectrometry after acid extraction. Two certified reference materials were analyzed and confirmed the accuracy of the method performed for the determination of total mercury [34]. Ikem and Egbeor used DMA for the determination of trace amount of mercury in canned fishes (mackerel, tuna, salmon, sardines and herring). The calibration of the equipment was performed using a certified reference material of dogfish muscle. The analysis of two certified reference materials demonstrated accuracy of this method [35]. Carbonell et al. employed the DMA method proposed for the quantification of total mercury and methylmercury contents in the muscle of seawater fish. Total mercury content is determined directly in the fish muscle and methylmercury after an extraction step using microwave assisted extraction in acidic conditions with toulene [36]. Tsiaib et al. determined the total mercury content in 69 red mould rice (Monascus) food samples purchased in Taipei, Taiwan using DMA. During the validation studies the accuracy was confirmed by analysis of a certified reference material of apple leaves. Addition/recovery tests were also performed and the average recovery was 95% [37]. Martins et al. established a method employing DMA for the quantification of total mercury content in 87 infant food samples purchased in Lisbon City, Portugal. The accuracy evaluation involved analysis of
certified reference materials of skimmed milk powder, rice, spinach, chicken, strawberry leaves and oyster tissue [38]. A method proposed by Torres and Martins-Teixeira determined the total mercury content in two types of fresh fish samples employing DMA. In it, analytical curves were performed using external calibration with aqueous standard solutions [39]. Vieira et al. proposed a method for the analysis of rice samples. Firstly, the samples are heated at 60 °C for 20 min in closed system and, after, the samples are mixed with potassium bromide and potassium bromate (fl ow injection system). This system causes reagent and condensation of the volatile species, mercury is quantitatively transferred from solid phase to liquid phase of the suspension and it is determined by CV AAS [9]. Several analytical techniques have been employed for the determination of mercury in food matrices using slurry sampling, however the procedures which used ETV ICP-MS [43–46] and CV ICP-MS [47] provided have lower limit of quantification. During the determination of mercury in cereals using slurry sampling and fl ow injection chemical vapor generation as the sample introduction system in ICP-MS, mercury was quantified by isotope dilution technique because the external calibration technique cannot be used [47]. Cava-Montesinos et al. proposed a method employing cold vapor atomic fl uorescence for the determination of mercury in milk by slurry sampling using multicommutation. Several chemical reagents were used for the slurry preparation including potassium bromide and potassium bromate [48]. Segade and Tyson proposed two simple fl ow injection (FI) systems for mercury speciation analysis in fish tissue samples by slurry sampling cold vapor atomic absorption spectrometry [49]. Chen and Jiang developed a method employing slurry sampling electrothermal vaporization inductively coupled plasma mass spectrometry for the determination of arsenic, selenium and mercury in fl sh samples. Palladium and thiocetamide were used as modifi ers. The quantifi cation of the elements was performed using analyte addition technique [50]. Table 2 shows the analytical parameters of methods developed for the determination of mercury in food matrices employing slurry sampling.

2.3. Solid sampling

Direct analysis employing the solid sampling technique has great advantages over sample digestion procedures especially for the determination of mercury because of the volatile character of this element and its species. Solid sampling allows acceleration and simplifi cation of the analytical procedures of sample preparation with sensitivity due to the absence of any dilution, and it avoids the use of chemical reagents and frequently allows quantifi cations employing the external calibration technique using aqueous standards. However, the drawbacks of this technique are: the accuracy is very infl uenced by sample homogeneity and low reproducibility. The solid sampling has been utilized for the determination of mercury in several matrices such as: contaminated soils [51,52], airborne particulate matter [53], polymers [54], and biological samples [54,55], but its application for food analysis has been limited. Methods have been established for the determination of mercury in fl sh [56], tuna fl sh [57], milk powder [57], spinach [57], seafood [58] and fl sh [59]. Table 3 shows the analytical parameters of methods proposed for the determination of mercury in food samples.

3. Strategies involving sample digestion for the determination of mercury in food matrices

3.1. Closed vessels and thermal heating

Acid digestion bombs are closed systems that are heated by convection using a conventional oven. In this, the sample amount used for digestion is rigorously controlled because of the risk of explosions. In the past, this digester system was widely recommended for sample mineralization during the determination of volatile species including mercury [60,61].

3.2. Cold finger

The sample mineralization utilizing the refl ux system “cold finger” [62] is one of the strategies that have been recently employed for the sample preparation during the determination of volatile chemical elements: lead [63,64] and others [65–67]. The “cold finger” is a glass tube in the shape of a fi nger, which is placed over the digester tube during the acid digestion by conventional heating using block digester [62]. This system causes refl ux and condensation of the volatile species, avoiding the loss by evaporation. Advantages, drawbacks and use of this refl ux system during the digestion of inorganic and organic matrices are summarized in a review paper [62]. The “cold finger” system has been used in sample preparation procedures for the determination of mercury in sediment samples [68], biological matrices [69], and crude oil and related products [70]. However, it has not reported any work involving digestion of food samples for the determination of mercury.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample mass (g)</th>
<th>LOD</th>
<th>LOQ</th>
<th>RSD</th>
<th>Calibration strategy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuts</td>
<td>0.1</td>
<td>0.08 μg kg⁻¹</td>
<td>0.3 μg kg⁻¹</td>
<td>2–14%</td>
<td>ECT</td>
<td>[33]</td>
</tr>
<tr>
<td>Mussel</td>
<td>0.1</td>
<td>0.01 ng</td>
<td>&lt;0.1 ng</td>
<td>&lt;1.5%</td>
<td>ECT</td>
<td>[34]</td>
</tr>
<tr>
<td>Canned fish samples</td>
<td>0.02 ng</td>
<td>0.24 μg kg⁻¹</td>
<td>&lt;14.79%</td>
<td>ECT</td>
<td>[35]</td>
<td></td>
</tr>
<tr>
<td>Fish muscle</td>
<td>0.05–0.10</td>
<td>0.6 μg kg⁻¹</td>
<td>&lt;1.5%</td>
<td>ECT</td>
<td>[35]</td>
<td></td>
</tr>
<tr>
<td>*Rice</td>
<td>0.1–0.5</td>
<td>0.10 μg kg⁻¹</td>
<td>0.29 μg kg⁻¹</td>
<td>&lt;1.0%</td>
<td>ECT</td>
<td>[38]</td>
</tr>
<tr>
<td>Infant foods</td>
<td>0.1</td>
<td>0.10 μg kg⁻¹</td>
<td>3.0 μg kg⁻¹</td>
<td>&lt;4.4%</td>
<td>ECT</td>
<td>[39]</td>
</tr>
<tr>
<td>Fishes</td>
<td>0.1</td>
<td>2.5 ng g⁻¹</td>
<td>2.7–3.4%</td>
<td></td>
<td></td>
<td>[40]</td>
</tr>
</tbody>
</table>

ECT = external calibration technique.

* Foods from Taiwan containing red mould rice (Monascus).

Table 1
Analytical parameters of methods developed for the determination of mercury in food samples using DMA.
3.3. Microwave heating

Microwave heating enables a rapid decomposition of samples due to a control over the time and heating power. Microwave digestion can be performed in open, closed, and flow systems. Open microwave systems have the risk of escape of volatile components of the sample, which may influence greatly the results. However, this type of system allows removal of gaseous products of the reactions and also the addition of reagents during the digestion process. Closed microwave systems are simpler and faster than open systems and their use reduces the risk of contamination. However, this type of device allows the use of only small amounts of sample, due to the large amount of gases released and the consequent risk of explosion. In microwave assisted extraction, the critical experimental factors are the concentration and volume of the extractor reagent, irradiation temperature, extraction time, sample mass and stability of the species [71,72]. Extraction procedures using dilute acid combined with solvent extraction have been proposed solid samples such as food, soil and sediments. Nitric acid, hydrochloric acid and hydrogen peroxide reagents have been used in the digestion procedures.

Alkaline solution of tetramethylammonium hydroxide was used in the digestion procedure of a method proposed for the determination of methylmercury in fish employing gas chromatography–mass spectrometry. Digestion conditions were optimized and the results obtained demonstrated that the analytical signals were strongly dependent of the heating time and irradiating power. Long heating times or high irradiating power resulted in low content of methylmercury [73]. Tuzen used nitric acid and hydrogen peroxide in a microwave digestion system for the determination of mercury and other trace elements in fish samples [74]. De Paz et al. also employed hydrogen peroxide and nitric acid in a digestion procedure of fish samples using a microwave oven for the determination of mercury by flow injection analysis system cold vapor atomic absorption spectrometry [75]. Hight and Cheng used a mixture of nitric acid and sodium chloride in a microwave oven during the digestion of seafood samples for the determination of total mercury by CV AAS [76]. Drennan-Harris et al. established a comparison between microwave digestion and acid extraction procedures proposed for the determination of mercury in rice samples using ICP-MS. They concluded that both procedures are effective for rice digestion [77]. Carrasco and Vassileva proposed an analytical procedure for the determination of methylmercury in marine biota samples using gas chromatography coupled to atomic fluorescence spectrometry. Five different extraction procedures were tested, including acid and alkaline leaching assisted by microwave and conventional oven heating and also enzymatic digestion. The highest extraction recoveries were obtained using alkaline extraction (potassium hydroxide in methanol), microwave-assisted extraction employing hydrochloric acid and enzymatic digestion with protease XIV [78]. A method was proposed for the determination of arsenic, cadmium, chromium, lead and mercury in seafood species used for sashimi. The mineralization procedure of the samples was performed in a closed microwave digestion system utilizing 7 mL of concentrated nitric acid and 3 mL of hydrogen peroxide. The elements were determined by ICP OES [79]. Kosanovic et al. quantified cadmium, mercury, lead, arsenic, copper, and zinc in human breast milk by ICP-MS. The sample mineralization procedure involved use of a microwave oven [80]. A sample preparation procedure for the determination of mercury in breast milk was established using 1 mL of homogenized milk sample, 2 mL Suprapur nitric acid and a closed microwave oven. The digested sample obtained was transferred to a 25 mL volumetric flask utilizing ultrapure water and the total mercury was determined by AFS using stannous chloride as reducing agent [81]. Koplik et al. proposed a method for the determination of methylmercury and inorganic divalent mercury in fish, vegetables, herbs and cereals using reversed-phase liquid chromatography hyphenated with inductively coupled plasma mass spectrometry. A procedure involving an extraction step of the mercury species using a mercaptoethanol acid solution was efficient for the quantification of methylmercury and inorganic divalent mercury in fish muscle and vegetable tissues [82]. Qin el.

### Table 2
Analytical characteristics of methods developed for the determination of mercury in food samples using slurrying.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample mass (g)</th>
<th>Homogenization</th>
<th>Size (μm)</th>
<th>Vapor generation</th>
<th>Analytical technique</th>
<th>Precision</th>
<th>LOQ</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbs</td>
<td>0.5</td>
<td>Ultrasonic bath</td>
<td>150 –</td>
<td>–</td>
<td>ETV-ICP-MS</td>
<td></td>
<td>4.0</td>
<td>0.3 ng g⁻¹</td>
<td>[43]</td>
</tr>
<tr>
<td>Rice and Ficus microcarpa leaves</td>
<td>1.0</td>
<td>Ultrasonic bath and vortex mixer</td>
<td>150 –</td>
<td>–</td>
<td>ETV-ICP-MS</td>
<td></td>
<td>10</td>
<td>2.6 ng g⁻¹</td>
<td>[44]</td>
</tr>
<tr>
<td>Rice flour</td>
<td>0.1</td>
<td>Ultrasonic bath</td>
<td>100 –</td>
<td>–</td>
<td>ETV-ICP-MS</td>
<td></td>
<td>10</td>
<td>2.6 ng g⁻¹</td>
<td>[45]</td>
</tr>
<tr>
<td>Rice flour</td>
<td>0.2</td>
<td>Ultrasonic bath</td>
<td>100 –</td>
<td>–</td>
<td>ETV-ICP-MS</td>
<td></td>
<td>10</td>
<td>1.75 ng g⁻¹</td>
<td>[46]</td>
</tr>
<tr>
<td>Rice and wheat flour</td>
<td>0.6</td>
<td>Ultrasonic bath heated (85 °C)</td>
<td>–</td>
<td>2.25% (w/v) NaBH₄</td>
<td>CV-ICP-MS</td>
<td></td>
<td></td>
<td>0.23 ng g⁻¹</td>
<td>[47]</td>
</tr>
<tr>
<td>Milk</td>
<td>2</td>
<td>Ultrasonic bath</td>
<td>–</td>
<td>3 mol L⁻¹ HCl 2.5% (w/v) SnCl₂</td>
<td>CV AFS</td>
<td></td>
<td>3.4</td>
<td>0.036 ng</td>
<td>[48]</td>
</tr>
<tr>
<td>Fish</td>
<td>0.25</td>
<td>Magnetic stirrer</td>
<td>100 –</td>
<td>0.75% (w/v) NaBH₄</td>
<td>FI-CV AAS</td>
<td></td>
<td></td>
<td>2.49</td>
<td>221 g ng⁻¹</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td>ETV-ICP-MS</td>
<td></td>
<td></td>
<td>112</td>
<td>11 ng g⁻¹</td>
<td>[50]</td>
</tr>
</tbody>
</table>
determined total mercury in fishes employing ICP-MS. The sample mineralization was performed using nitric acid and hydrogen peroxide in a microwave oven [83]. Zhao et al. proposed a method for the speciation analysis of mercury in fish samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. The mercury species were extracted using a 1 M hydrochloric acid solution in a microwave digester [84]. The efficiency of eight different analytical procedures commonly used for the extraction of mercury species in biological samples was evaluated by the analysis of a certified reference material of fish used for the determination of total mercury and methylmercury [85]. The extraction procedures tested were: alkaline extraction with KOH or tetramethylammonium hydroxide; acid leaching with hydrochloric, nitric or acetic acid; extraction using L-cysteine hydrochloride; and enzymatic digestion with protease XIV. Total mercury and mercury species from all extractions were determined using ICP-MS and HPLC–ICP-MS, respectively. Procedures using alkaline digestion with microwave assisted extraction and ultrasonic assisted extraction were the most efficient, where the levels of transformation of mercury species were the lowest (6% or less). Extraction using 5.0 mol L\(^{-1}\) hydrochloric acid and/or enzymatic digestion with protease also showed good results. The authors warn that despite the frequent use of acid leaching for the extraction of mercury species in fish samples, the lowest extraction efficiencies and greater transformation between mercury species were obtained when microwave assisted extraction with 4.0 mol L\(^{-1}\) nitric acid or acetic acid was used [85]. An efficient method was proposed for the speciation analysis of mercury in fish tissue using microwave assisted extraction in a closed system. Quantitative extraction of mercury species was obtained by leaching using 5.0 mol L\(^{-1}\) hydrochloric acid and 0.25 mol L\(^{-1}\) sodium chloride for 10 min at 60 °C. Total mercury and mercury species were determined using ICP-MS and HPLC–ICP-MS, respectively [86]. An alternative procedure using a vessel that allows partial release of gases was reported. This device enabled an increase of sample mass, the completeness of oxidation, and the retention of volatile elements in solution [87]. Table 4 summarizes the analytical parameters of procedures proposed for the determination of mercury in food samples employing microwave assisted digestion.

3.4. Vigreux column

In 1990, Landi et al. used the Friedruchs and Vigreux condensers in sample preparation procedures during the determination of mercury in vegetable matrices employing CV AAS [95]. In another method, these authors employed potassium dichromate, diluted sulphuric acid and a reflux system using a condenser for the determination of total mercury in seafood and other protein-rich products by CV AAS [96]. Subsequently, a sample digestion procedure using the Vigreux column was proposed for the determination of mercury in core sediments by cold vapor atomic fluorescence spectroscopy [97]. In the last times, this same research group has established sample preparation procedures using Vigreux column and oxidant acid mixtures for the determination of mercury in foods such as: vegetables [98], teas [99], meals [100] and other matrices [101,102] employing square wave anodic stripping voltammetry (SW ASV) using a gold electrode.

4. Other sample preparation procedures for the determination of mercury in food samples

Although NAA presents great advantages for the determination of mercury, employing this technique for the analysis of food is relatively low. Singh and Garg determined the content of 16 trace elements in Indian cereals, vegetables and spices employing NAA. The sample preparation procedure for neutron irradiation of the seed matrices (rice, wheat fenugreek and black pepper) involved cleaning with tissue paper, followed by drying using an IR lamp at 80 °C and afterward, grinding to powder using a food processor/mixer and an agate mortar. The vegetable samples were washed with distilled water and dried in an oven at 80 °C overnight. Then, these matrices were grinded to powder in an agate mortar [103]. Rizzo et al. determined total mercury in the muscle and liver of four fish species by NAA. For irradiation, the samples were dried, homogenized and sealed in quartz ampoules. These authors warn that the limits of detection and quantification for mercury in NAA determinations depend strongly on the analytical conditions of the analyzed sample, particularly the gamma-ray spectral background generated by other elements contained in the sample [104]. Anderson evaluated the effect of L-cysteine on drying and neutron irradiation of fish muscle and liver of four fish species from fish tissue. During the experiments some tissue samples were dried and analyzed by NAA. Other samples were treated with L-cysteine followed by freeze drying and also analyzed by NAA. The results for the untreated tissues showed that mercury may be largely lost (23–49%) by volatilization during irradiation. Treatment with L-cysteine reduced significantly these losses; however, these reductions were highly dependent of the drying procedure used. Freeze drying of treated tissues at −50 °C and 1.3–2.7 Pa was most efficient (1.0–3.5%) [105]. Antoine et al. determined mercury and several other elements in twenty-five rice brands that originated from Jamaica using NAA. For neutron irradiation the rice samples were weighed out into pre-cleaned double polyethylene bags and heat sealed in pre-cleaned polyethylene vials [106].

Analytical strategies involving microwave-induced combustion (MIC) have been considered as a good alternative for the sample preparation during the determination of metals and non-metals in complex matrices [107,108]. This technique has as advantage the possibility to digest relatively large amounts of sample (up to 500 mg) using low volumes of acids, allowing a final solution with a low residual carbon content. So, MIC has been also employed for the sample digestion during the determination of mercury in fish [109], soil [110], coal [111] and pharmaceutical ingredients [112].

Schmidt et al. evaluated the influence of drying conditions on the behavior of mercury species (inorganic mercury and methylmercury) present in six different fish species. The drying conditions studied were as follows: air circulation drying oven in different temperatures (50 to 175 °C) and lyophilization (0.25 mm Hg. −2 °C). The evaluation of the results was based on losses and conversions of original Hg species after each drying condition. The determination of total mercury and its species were performed employing CVG ICP-MS and LC–CVG–ICP-MS, respectively. The results demonstrated that for drying temperatures above 100 °C, losses and conversions of methylmercury to inorganic mercury can occur for some fish species [109].

Marshadizadeh et al. proposed a pre-concentration procedure by magnetic solid phase extraction using Fe\(_3\)O\(_4\) nanoparticles coated with 3-(trimethoxysilyl)-1-propanethiol and modified with ethylene glycol bis-mercaptoacetate for the determination of mercury in food samples (rice, tuna fish and tea leaves) employing CV AAS as analytical technique [113]. Lemos and dos Santos employed a pre-concentration system containing a mini-column packed with the Amberlite XAD-4 sorbent functionalized with 2-(2′-benzothiazolylazo)-p-cresol (BTAC) for the determination of mercury in fish, shellfish and saliva using CV AAS [114]. An enrichment procedure employing Fe\(_3\)O\(_4\) magnetic nanoparticles functionalized with dithizone was investigated for the extraction and determination of ultra-trace amounts of mercury in table salt, green tea, vegetables, toothpaste, and water samples using CV AAS [115]. A pre-concentration procedure for the determination of trace amount of mercury in sea and river fish samples was proposed using dispersive liquid–liquid micro-extraction [116]. A procedure for the determination of total mercury in the muscle tissue of fish was developed using GF AAS. Copper nitrate and sodium tungstate were tested as chemical modifier and permanent modifier, respectively. A limit of quantification of 0.047 mg kg\(^{-1}\) was obtained [117].

A multisyringe flow injection system was established for the determination of total mercury in rice samples using cold vapor atomic
with toluene and back-extraction into an aqueous L-cysteine solution. The quantification was performed sequentially with stannous chloride and sodium tetrahydroborate for the determination of methylmercury in food. For speciation, the digested samples in alkaline conditions were reduced by mercury amalgamation and atomic absorption has been performed by gas chromatography–mass spectrometry; FIAS-CV-AAS: flow injection analysis system cold vapor atomic absorption spectrometry; AFS: cold vapor atomic fluorescence spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; CV AAS: cold vapor atomic absorption spectrometry; FIAS-CV-AAS: flow injection analysis system cold vapor atomic absorption spectrometry; AFS: cold vapor atomic fluorescence spectrometry.

A non-chromatographic method was developed for the determination of methylmercury and inorganic mercury in the muscle tissues of ten freshwater fish species employing CV AAS as analytical technique. For speciation, the digested samples in alkaline conditions were reduced sequentially with stannous chloride and sodium tetrahydroborate for the quantification of inorganic mercury and methylmercury, respectively. A procedure involving sample thermal decomposition followed by mercury amalgamation and atomic absorption has been performed for the determination of methylmercury in fish. An extraction step with toluene and back-extraction into an aqueous L-cysteine solution allows the separation for the speciation analysis. An on-line pre-concentration system with valves electronically controlled using a minicolumn packed with polytetrafluoroethylene was developed for the determination of mercury in water samples employing CV AAS. In it, mercury(II) ions as pyrrolidinedithiocarbamate complexes are firstly pre-concentrated on the minicolumn and afterward these complexes are eluted and reduced using a sodium tetrahydroborate(III) solution. A pre-concentration factor of 35 and a limit of detection of 0.02 μg L⁻¹ were obtained. Goudarzi et al. determined mercury, lead and cadmium in breast milk of healthy lactating women from Iran. The sample preparation step involved a volume of 25 mL of milk sample and digestion in semi-closed glass apparatus in the presence of 7 mL concentrated nitric acid and 3 mL of hydrogen peroxide. After cooling, the volume was adjusted to 50 mL using ultrapure water and mercury as quantified employing CV AAS.

Procedures involving amalgamation in gold traps or Pd-based substrates have been described for the preconcentration of mercury after cold vapor generation. Pd-based substrates, such as Pd wire, Pd-coated SiO₂ fiber and Pd-coated stainless steel wire have also been applied for the preconcentration of Hg using solid-phase microextraction methodology. Hg vapor generation is carried out in a vial and the volatile Hg formed is then amalgamated onto the Pd-based materials. The preconcentration procedures based on the trapping of mercury on a gold filament preconcentrator are followed by rapid thermal desorption and detection. The absorption of mercury in a gold surface is highly effective and well known. This phenomenon usually generates selective procedures because mercury is the only species absorbed if gas acids are removed. Preconcentration using mercury amalgamation in gold trap has been applied to the determination of the element in fish tissues, water and biological matrices.

### Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample amount</th>
<th>Reagent for digestion</th>
<th>Power (W)</th>
<th>Time of irradiation (min)</th>
<th>Detection technique</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>1.0 g</td>
<td>6.0 mL of concentrated HNO₃ (65%), 2.0 mL of concentrated H₂O₂ (30%) and 2.0 mL of H₂O</td>
<td>250</td>
<td>2.0</td>
<td>CV AAS</td>
<td>[74]</td>
</tr>
<tr>
<td>Fish</td>
<td>0.1–0.4 g</td>
<td>4.0 mL of concentrated HNO₃ (65%) and 0.2 mL of concentrated H₂O₂ (30%)</td>
<td>300</td>
<td>4.0</td>
<td>FIAS-CV</td>
<td>[75]</td>
</tr>
<tr>
<td>Seafood</td>
<td>0.1 mg</td>
<td>1.0 mL of 1.0 % w/v NaCl and 5.0 mL of concentrated HNO₃ (65%)</td>
<td>1200</td>
<td>20.0</td>
<td>CV AAS</td>
<td>[76]</td>
</tr>
<tr>
<td>Rice</td>
<td>0.5 g</td>
<td>200 μg of L-cysteine, 3.0 mL of H₂O and 3.0 mL of concentrated HNO₃ (65%)</td>
<td>400</td>
<td>20.0</td>
<td>ICP-MS</td>
<td>[77]</td>
</tr>
<tr>
<td>Breast milk</td>
<td>1.0 mL</td>
<td>2.0 mL of concentrated HNO₃ (65%)</td>
<td>–</td>
<td>–</td>
<td>CV AFS</td>
<td>[78]</td>
</tr>
<tr>
<td>Duplicate meals (breakfast and lunch) and foodstuffs of animal origin</td>
<td>0.2 and 0.5 g (dry samples) or 0.5 and 1.0 g (wet samples)</td>
<td>3.0 mL of concentrated HNO₃ (65%)</td>
<td>500², 800³, 1000⁴</td>
<td>20.0</td>
<td>ICP-MS</td>
<td>[88,89]</td>
</tr>
<tr>
<td>Dietary supplements</td>
<td>0.5 g</td>
<td>10.0 mL of concentrated HNO₃ (65%)</td>
<td>100⁴, 50⁴, 20⁴</td>
<td>15.0</td>
<td>Hg analyser</td>
<td>[90]</td>
</tr>
<tr>
<td>Fish</td>
<td>0.2 g</td>
<td>8 mL of concentrated HNO₃ and 2 mL of concentrated H₂O₂</td>
<td>25⁵, 180⁶</td>
<td>15</td>
<td>ICP-MS</td>
<td>[92]</td>
</tr>
<tr>
<td>Fish</td>
<td>0.25 g</td>
<td>5.0 mL of concentrated HNO₃ (65%) and 0.5 mL of concentrated H₂O₂ (30%)</td>
<td>75⁷, 180⁶</td>
<td>15</td>
<td>ICP-MS</td>
<td>[92]</td>
</tr>
<tr>
<td>Rice</td>
<td>0.50 g</td>
<td>2.0 mL of concentrated HNO₃ (65%), 1.0 mL of concentrated H₂O₂ (30%) and 5.0 mL of H₂O</td>
<td>5.0, 500⁶, 5.0, 4.0, 2.0, 100⁶, 100⁶, 100⁶</td>
<td>15</td>
<td>ICP-MS</td>
<td>[93]</td>
</tr>
<tr>
<td>Rice</td>
<td>0.5 g</td>
<td>4.0 mL of concentrated HNO₃ (65%), 2.0 mL of concentrated H₂O₂ (35%) and 4.0 mL of H₂O</td>
<td>RT-85⁶, 85-145⁶, 145-180⁶, 180⁶, 180-30⁶</td>
<td>3.0</td>
<td>CV AFS</td>
<td>[94]</td>
</tr>
</tbody>
</table>

TMAH: tetramethylammonium hydroxide; GC-MS: gas chromatography–mass spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; CV AAS: cold vapor atomic absorption spectrometry; FIAS-CV-AAS: flow injection analysis system cold vapor atomic absorption spectrometry; AFS: cold vapor atomic fluorescence spectrometry.

| a Initial.  | b Final. | c 100% power. | d Pressure, psi. | e Ventilation. |

### 5. Sample preparation for the speciation analysis of mercury in food samples

The determination of organic mercury in food has been frequently reported in the literature, due to the high toxicity of these species...
Various chromatographic techniques have been employed for the quantification of mercury species, including gas chromatography (GC) and high-performance liquid chromatography (HPLC) [130]. The hyphenation with spectrometric detection techniques, such as atomic fluorescence spectrometry (AFS), atomic emission spectrometry (AES), atomic absorption spectrometry (AAS) and mass spectrometry (MS) has resulted in powerful tools for the speciation of organic mercury species in various samples such as red wine [131], fish [132–134], rice [135] and seafood [136]. Capillary electrophoresis has also been used in some applications. The determination of methylmercury in fish was performed by leaching with HBr analyte extraction with toluene and back-extraction with aqueous l-cysteine [119]. MeHg was determined using sample thermal decomposition followed by mercury amalgamation and atomic absorption. A method for the speciation of mercury in seafood used the hyphenation of LC–ICP-MS and a fast sample preparation procedure [136]. Mercaptoethanol, l-cysteine and HCl were used to extract the mercury species, with the aid of sonication for 15.0 min. A C8 reverse phase column has been used for the separation of mercury species. Organic mercury and inorganic mercury were determined in fresh and canned fish and in blood using a method based on ICP-MS coupled with high performance liquid chromatography (HPLC) [137]. Gas chromatography with atomic emission detection has been used in a simple method for the speciation of mercury species in fish and seafood [138]. Methylmercury was measured in rice (Oryza sativa L.) using gas chromatography (GC)–CVAFS detection. Cereal was grown in soils from areas of abandoned mercury mines [135]. Flow injection cold vapor generation–inductively coupled plasma mass spectrometry (FI-CVG–ICP-MS) and gas chromatography–ICP-MS (GC–ICP–MS) have been applied for the determination of total Hg, inorganic Hg and methylmercury in red wine [131]. Hight and Cheng proposed a method for the determination of methylmercury and total mercury in seafood samples [139]. In it, mercury compounds are extracted from the samples using 50 mL of an aqueous solution containing V l-cysteine and hydrochloric acid followed by heating for 120 min at 60 °C. Afterward, the mercury compounds containing the aqueous extracts are separated by reversed-phase high performance liquid chromatography using a C-18 column and detected employing inductively coupled plasma–mass spectrometry at mass-to-charge ratio 202. Methyl mercury and inorganic mercury are determined in extracts. Montero-Alvarez et al. developed a method for the speciation analysis of mercury in edible fish samples employing HPLC–ICP-MS and isotope dilution analysis. The extraction of the mercury species was performed by mercaptoethanol, l-cysteine, and hydrochloric acid and sonication for 30 min [140]. Huang et al. proposed a procedure for the determination of methylmercury and total mercury in marine fish samples (five species). Methylmercury was quantified employing HPLC–AFS and total mercury using a Direct Mercury Analyzer. Methylmercury was extracted using microwave radiation [141].

6. Conclusions

The development of methods for the determination of trace amounts of mercury in food samples is still important and timely considering the high toxicity of this element and its compounds. During the determination of mercury in food samples the quantification step is very simple and can be done using CV AFS or CV AAS, which are analytical techniques highly sensitive for mercury with low cost. The ICP-MS and CV ICP-MS techniques are also often used but the analytical cost is very high. However, the sample preparation step is very critical because of the volatile character of mercury and its compounds. Thus, further studies should be made considering the variety of food matrices that may contain this element.

The employment of the Direct Mercury Analyzer (DMA) has been a good option for the direct determination of mercury in samples of solid and liquid foods. This enables the analysis without the use of chemical reagents and it allows the use of external calibration technique using certified reference materials or aqueous standards.

Slurry sampling is a sample preparation technique that has been also employed for the determination of mercury in food samples. In this, the sample amount is not limited as DMA and/or solid sampling technique and the calibration can be performed eventually by external calibration technique using aqueous standards. Procedures have been established by chemical vapor generation such as: CV AAS, CV AFS, CV ICP-MS and also using electrothermal vaporization-inductively coupled plasma mass spectrometry (ETV-ICP-MS), which allows low limits of detection and quantification. Foods of animal and vegetable origins have been analyzed by this technique.

The reflux system using “cold finger” has been often employed for the sample preparation during the determination of volatile elements. Several methods for the determination of mercury in complex matrices such as: crude oil and sediment and biological samples have been developed using this digestion strategy. However, there is no work involving sample preparation using cold finger for the determination of mercury in food samples. Studies carried out with other volatile elements in food matrices and the works established for mercury suggest that this technique of reflux may be used for the mineralization of food samples for the determination of mercury.

Microwave assisted digestion (MAD) in closed vessels has been widely applied for the digestion of many food matrices during the determination of mercury, due to its high efficiency and reduced risks of losses and contamination in comparison with conventional digestion procedures using open vessels. For the determination of total mercury, the reagents most often used are: nitric acid, hydrochloric acid and hydrogen peroxide. Alkaline digestions using tetramethylammonium or potassium hydroxide have been recommended by several authors for the determination of methylmercury.

The speciation analysis of mercury in food samples has been performed employing gas chromatography (GC) and high-performance liquid chromatography (HPLC) as separation techniques, being the detection established by several spectroanalytical techniques such as: atomic fluorescence spectrometry (AFS), atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS).

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References


