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Review Article

Recent updates on analytical methods for mercury determination: A review

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Abstract

Mercury is a heavy metal that can act as a contaminant that can be highly toxic. Exposure to mercury can pose a risk of central nervous system issues, memory loss, tremors, convulsions, sleeplessness, irritability, excessive fear, brain damage, paralysis, psychosis, and decreased self-confidence. Therefore, it is critical to provide trustworthy analytical methods for mercury detection in order to monitor the level of mercury. The purpose of this review is to offer critical perspectives on analytical techniques that can be used to determine mercury levels in environmental and biological samples. This current review focuses on the latest analytical methods that have been used for mercury determination in recent years. This review reveals that atomic absorption spectroscopy (AAS), liquid chromatography (LC), and inductively coupled plasma (ICP) are still routinely used to detect mercury levels. Spectrophotometric, immunoassay, electrochemical, and colorimetric testing methods are still being developed.

Keywords

chromatography, electrochemical, environmental monitoring, mercury determination, spectroscopy

Introduction

Heavy metals are naturally occurring elements with high atomic weights and densities and can be considered one of the most dangerous contaminants (Tchounwou et al. 2012; Abu Shmeis 2022). Heavy metals can interfere with the function of biomolecules such as proteins and enzymes due to their ability to bind with several functional groups, including carboxylic acid, amino, and sulfur-containing groups. Moreover, they have the ability to precipitate phosphate biomolecules or accelerate their breakdown (Tamás et al. 2014). A person who is exposed to mercury exceeding the maximum limit will experience central nervous system disorders, memory loss, tremors, convulsions, insomnia, decreased self-confidence, irritation, excessive fear, brain damage, paralysis, slurred speech, and delirium (Bernhoft 2012; WHO 2021).

Mercury has the chemical symbol Hg (Hydragryum). It is a heavy metal with atomic number 80 and a molecular mass of 200.59 g/mol. Mercury dissolves easily in sulfuric acid and is resistant to alkaline pH conditions. The density of mercury is 13.5 g/cm3, which is the highest density compared to all liquid objects. Mercury metal has a heavy, silvery-white color and is odorless at room temperature (National Center for Biotechnology Information 2023). It exists in liquid form at room temperature with good electrical conductivity, but also easy to evaporate at room temperature. Mercury has the weakest metallic bonds

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when compared to other metals. The high vapor pressure at room temperature is due to its weak metallic bonds. This has a dangerous impact because it causes the inhalation of mercury vapor by living organisms, which will result in death. Although it is dangerous when inhaled, metallic mercury is not easily absorbed into undamaged skin. Mercury is also able to dissolve various metals to form alloys or amalgams (Bernhoft 2012; National Center for Biotechnology Information 2023).

Mercury emissions into the atmosphere were primarily caused by various industrial processes, including coal-burning, production of cement, iron and steel production, waste disposal, and direct production of mercury (Wang et al. 2020). Mercury is emitted in its elemental form (Hg0) and deposited to soil and water as divalent mercury (Hg2+). Once deposited, it can undergo methylation to its organic form (methylmercury, MeHg) and accumulate in organisms such as marine animals (O'Connor et al. 2019). As seafood is the main protein source in many countries, bioaccumulation of MeHg in seafood may result in human mercury exposure (Lavoie et al. 2018). In 1956, an outbreak of mercury poisoning happened in Minamata City, Japan (Harada 1995). At least 439 deaths were reported, and thousands were afflicted with Minamata disease, which is associated with a set of neurological symptoms including ataxia, dysarthria, visual disturbances, hearing difficulties, and convulsions (Tamashiro et al. 1985). Findings suggested that this was caused by fish and shellfish consumption, which was contaminated with factory effluents discharged into the Minamata Bay (Basu et al. 2023). This incident drew the attention of the world to the danger of mercury exposure to human health. A study found that the median mercury level in the hair of Minamata residents was 30 ppm (Kessler 2013). WHO set the tolerable intake (PTWI) of inorganic mercury and methylmercury at 4 mg/kg body weight and 1.6 mg/kg body weight for human adults (FAO/WHO 2007, 2011). In the US, the limit for the level of mercury in bottled water and public drinking water is set by the FDA and US Environmental Protection Agency to 2 ppb (21 C.F.R 2022, 2023). The maximum mercury level for salt and supplements is 0.1 mg/kg products, and for seafood and fishery products, the limit depends on the species, which range from 0.3 to 1 mg/kg products (Commission Regulation 2023/915 2023).

A rising concern for the toxicity of mercury highlights the need to perform an analysis of mercury. Mercury is present in various samples, which warrants a specific pretreatment and analytical techniques. Suvarapu et al. (2013) found that cold vapor-atomic absorption spectroscopy (CV-AAS) and cold vapor-atomic fluorescence spectroscopy (CV-AFS) are the most common methods used by researchers for the determination of mercury. A newer study by the same authors (Suvarapu and Baek 2015) found that most of the studies focused on total mercury determination rather than performing speciation studies and that most researchers were concerned about the effect of interfering ions in the analysis of mercury. A more comprehensive review was conducted by Saleh et al. (Saleh et al. 2020), which provides a method of detection of mercury in gas, oil, and water phases, as well as the removal process. In recent years, advancements were made to develop new methods to analyze mercury in biological and environmental samples. However, to the authors' knowledge, in recent years, there have still been no studies that summarize and compare the analytical methods based on the analytical principles.

Atomic absorption spectroscopy, fluorescence spectroscopy, UV-Vis spectroscopy, inductively coupled plasma mass spectrometry, resonance scattering spectroscopy, and electrochemical methods are among the analytical methods that can be used to detect mercury (Clevenger et al. 1997; Karunasagar et al. 1998; Kopyść et al. 2000; Wen et al. 2010). However, there are newer methods that are still being developed to detect mercury in samples. This study aims to evaluate the literature on analytical techniques for mercury detection and offer critical perspectives on the benefits and drawbacks of each approach for assessing mercury in various samples, as well as the sample preparation required before performing analysis. In order to give a comprehensive review of applicable methodologies, we chose seminal works on the subject from more recent publications. After assessing the sample preparation techniques, we then discuss the analytical methods, including basic colorimetric assays, spectrometric, electrochemical, chromatographic, and miscellaneous methods, before concluding with some final thoughts.

Sample preparation

Mercury exists in various forms of matrices in the environment. Prior to the analysis, mercury must be released from the matrices to prevent interferences during the process (Fig. 1). This step is also pivotal to preconcentrating the mercury that is usually found in trace amounts in the environment. Commonly used sample preparation methods employ acid digestion, which involves acidification of the samples and the addition of another metal cation to outcompete ligand binding, which is sometimes followed by derivatization or complexation (Mester and Sturgeon 2003). Cysteine, thiosulfate, or thiol-containing reagents are usually involved in the process to facilitate phase transfer. Other methods that can be used for the extraction of mercury from samples include liquid-liquid extraction (LLE) (Abbasi et al. 2019), solid-phase extraction (SPE) (Panichev et al. 2014), and pyrolysis (Park et al. 2013). The distillation method has also been used, considering the volatile nature of mercury (Sadasiva et al. 2017). For extraction of mercury from biological samples, alkaline digestion can be used to dissolve the matrix (Mester and Sturgeon 2003). Current technology such as microwave heating, closed vessel and thermal heating, cold finger using a reflux system, and a Vigreux column can also be used (Ferreira et al. 2015). The microextraction approach has gained significant attention recent years due to its sustainability and alignment with the principles of

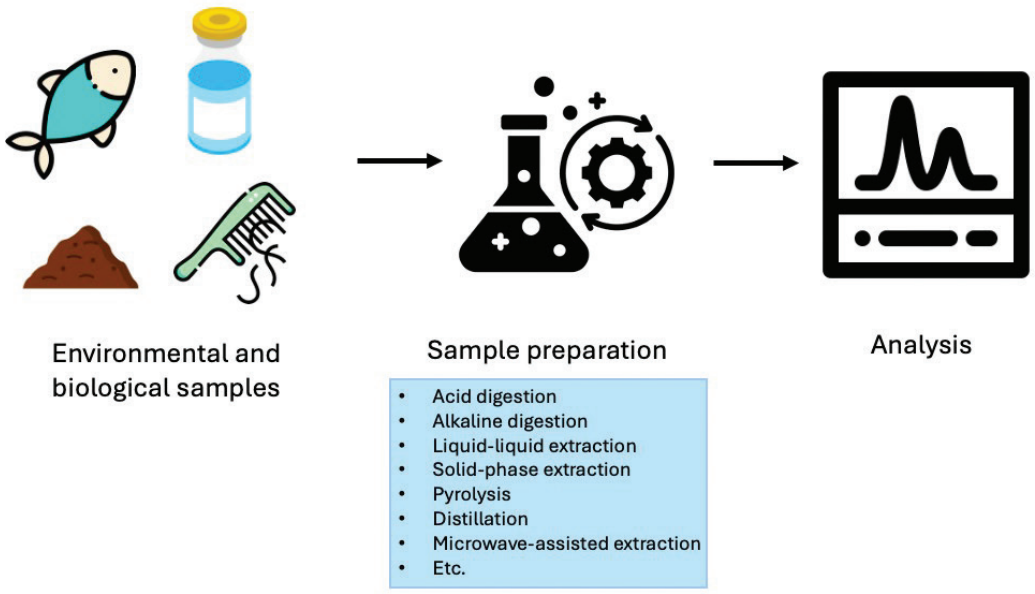

Figure 1. Process for mercury analysis.

green chemistry (Amico et al. 2022). Several studies have successfully used direct analysis of mercury without a pretreatment process and can be used in a wide range of matrices, but this method may be unsuitable for running large numbers of samples.

Analytical methods

The presence of mercury in the environment can harm humans because of the risk of toxicity and even death. To ensure safety, regulatory authorities establish maximum residue limits (MRLs) for mercury in various samples. Analytical techniques such as colorimetric assay, spectrophotometric, electrochemical analysis, and chromatographic analysis are used to detect and quantify mercury in samples. Novel miscellaneous techniques have also been developed in recent years, such as using deep eutectic solvent, resins, and polymers to preconcentrate the samples. Fig. 2 presents a compilation of analytical techniques for the examination of mercury in the sample, along with their respective advantages and disadvantages.

Colorimetric assay

A colorimetric assay is a method to detect analytes in a sample by causing a visible color change (Melikishvili et al. 2021). This approach relies on interactions between the analyte and the chromogenic reagent to cause a color shift. Colorimetric detection is appealing since it is straightforward, easy to read, and visible with the naked eye (Yang et al. 2020c). The simplicity of this method also allowed analysis to be conducted in situ without further sample preparation. Recently, a colorimetric detection method for mercury analysis was devised employing a paper-based sensor. Paper-based sensors are tools that contain chromogenic reagents and deposit the sample directly into the paper (Kuswandi et al. 2022). Paper-based sensors attracted attention due to their minimal development cost, widespread availability, and ability to conduct on-site analyses (Noviana et al. 2020). Several chromogenic reagents were utilized for mercury analysis, as shown in Table 1.

Microfluidic paper-based analytical devices (μPADs) and reagent-impregnated paper strips/devices are low-cost tools used for analysis without the need for complex equipment. To make μPADs, patterns are drawn on paper using hydrophobic materials to control fluid movement. Some paper-based devices for mercury detection use colorimetric methods and can detect mercury ions (Hg2+) through photographic analysis (Nashukha et al. 2021). Guo et al. used a paper-based fluorescence sensor to detect ion of mercury in environmental water samples by employing MoS2 quantum dots (MoS2 QDs) functionalized with boronic acid as a chromogenic reagent (Guo et al. 2020). The B-MoS2 QDs exhibit blue fluorescence at an excitation wavelength of 300 nm, which is quickly quenched in the presence of Hg2+. The test paper is prepared by submerging filter paper in the B-MoS2 QDs solution. After that, it is taken out and allowed to cure at ambient temperature. When exposed to a UV lamp, it fluoresces blue at 365 nm. The test paper's fluorescence will be partially muted in different amounts after adding Hg2+ at various concentrations and allowing it to react for five minutes. With a detection limit of 1.8 nmol/L, this work offers a quick and selective method for detecting mercury using 18 interfering ions (Guo et al. 2020). A similar study was conducted by Nashukha et al., using iodide to form a purple-colored complex with mercury, which can then be analyzed using software. This method can detect mercury levels ranging from 50 to 350 mg/L, with a detection limit of 20 mg/L. They successfully tested mercury levels in contaminated soil and water samples, consistent with results from traditional testing methods. (Nashukha et al. 2021).

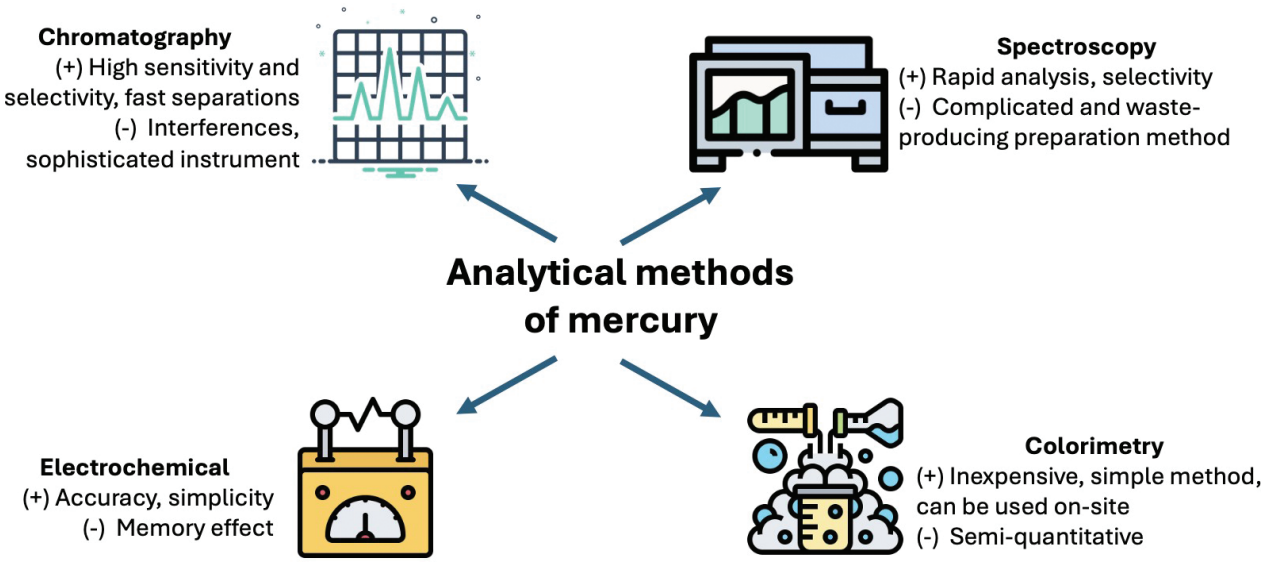

Figure 2. Analytical methods commonly used to analyze mercury.

Table 1. Colorimetric assay for mercury analysis.

No	Sample	Chromogenic reagent	Signal readout	Color change	LoD	Ref.
1	Water	Boronic acid-functionalized MoS2 QDs	Fluorescence (under UV lamp by naked eye)	Blue fluorescence	1.8 nmol/L	(Guo et al. 2020)
2	Soil	Nitric acid, sulfuric acid, deionized water	Microfluidic paper-based analytical devices (μPADs)	Analysis was done using software	50–350 mg L−1	(Nashukha et al. 2021).
3	River water	AgNPs ($AgNO_3$ with ascorbic acid as a reductant)	Smartphone application	Yellowish brown to colorless	0.86 ppb	(Firdaus et al. 2019)
4	Water	SERS-Active peroxidase-like Au@AgPt NPs	Colorimetric /SERS dual-mode detection	Colorless to blue	0.52 μM by naked eye 0.28 nM by SERS	(Song et al. 2020)
5	Water	Ag NPs-tryptophan nanoconjugate functionalized with 3-(trimethoxysilyl) propyl methacrylate (TMPM)	UV-Vis	Yellow to colorless	2 nM	(Balasurya et al. 2020)
6	Water	*Achillea wilhelmsii*-mediated AgNPs (Aw-AgNPs)	Smartphone application	Brown to colorless	0.30 × 10−6 M	(Mavaei et al. 2021)
7	Water	Green tea-mediated AgNPs	UV-Vis	Brown to colorless	–	(Prema et al. 2022)
8	Face cream	AgNPs-C. canephora fruit skin extract	UV-Vis	Dark brown to colorless	0.039 mg/L	(Sulistyart et al. 2023)
9	Water	Green tea-mediated AgNPs	UV-Vis	Brown to colorless	0.01 mg/L	(Patra et al. 2023)
10	Water	Bromelain enzyme (brn-AuNPs)	Image analysis	Red to blue	0.011 ppm	(Suhaidi et al. 2023)
11	Water	Cyanuric acid (CA-AuNPs)	Visual and/or ImageJ software	Pink to bluish purple	0.05 μM	(Saputri et al. 2023)

The current development of sensors is frequently based on nanoparticles, which are integrated with chromogenic reagents. Due to their size- and shape-dependent optical, chemical, and biological properties, nanoparticles formulated with noble metals such as gold (Au) and silver (Ag) have been developed widely, including for mercury monitoring (Grigorchuk 2018; Abdussalam-Mohammed 2020).

Using silver nanoparticles (AgNPs), Firdaus M. et al. created a portable analytical technique for mercury ion measurement (Firdaus et al. 2019). A smartphone was utilized to take digital photos that were then used for detection. Silver nitrate (AgNO3) was used to synthesize the AgNPs. Ascorbic acid was also used as a reductant in the synthesis. The addition of mercury ions led to a redox reaction, which caused the color change in the AgNPs from yellowish brown to colorless. The AgNPs were attached to a paper-based analytical device, and the "Mercury Detector" Android application was used for analysis. The limit of detection (LOD) for the proposed method was 0.86 ppb, which demonstrated a good sensitivity.

Song C et al. proposed a novel method using a combination of colorimetric and surface-enhanced Raman scattering (SERS) to achieve a rapid and sensitive detection of mercury (Song et al. 2020). A peroxidase-like Au@AgPt NP was synthesized by selectively depositing Pt on the edges of hexoctahedral gold (Au) nanoparticles. The surface of the Au@AgPt NPs was then modified with mercaptohexanol (MCH) to produce a colorimetric/SERS dual-mode probe (Au@AgPt@MCH). By monitoring the color change and SERS signal, the developed dual-mode

probe demonstrates that in genuine pure water containing Hg2+, colorimetric detection has a strong recovery (99.99%–102.55%) with RSDs less than 2.41%, and SERS detection has a recovery of 96.50%–99.50% with RSDs less than 5.75%. The test conducted on the river water sample shows good recovery with an RSD of less than 5%.

Another approach using nanoparticles for mercury detection was applied by Balasurya S et al. Colorimetric detection of mercury ion was done in ambient water samples with Ag NPs-tryptophan nanoconjugate combined with 3-(Trimethoxysilyl)propyl methacrylate (TMPM) to colorimetrically detect mercury ions in ambient water samples (Balasurya et al. 2020). The addition of Hg2+ ions to the Ag NPs-tryptophan nanoconjugate solution caused a color change from pale yellow to colorless. The limit of detection for this method was found to be 2 nM.

Despite the numerous advantages of nanoparticles, the method of producing AgNPs needed costly chemical raw materials and purification due to harsh reaction conditions and the need for reducing agents such as thiols, NaBH4, and NaOH (Pourmortazavi et al. 2008). Substantial effort was conducted to develop and apply ecologically benign methods for synthesizing AgNPs (Asariha et al. 2020; Mavaei et al. 2020). Green-synthesized AgNPs are non-toxic, affordable, renewable, and easy to handle (Lee and Jun 2019). Current advances focus on the development of a green synthesis method that uses natural resources, such as plant extract, as a reduction agent. Plants are the principal source of various metabolite compounds, which can increase the reaction time compared to the use of other natural resources like bacteria (Maarebia et al. 2019).

The development of green, sustainable silver nanoparticles has been studied extensively using various plant extracts, such as *Achillea wilhelmsii* (Mavaei et al. 2021), green tea (Prema et al. 2022), and robusta coffee (*Coffea canephora*) (Sulistyarti et al. 2023). The solution of the nanoparticles can be used directly in mercury ion detection or by integrating the nanoparticles into a paper to create a paper-based device. Mavaei et al. created a paper-based device by loading the paper with nanoparticles, which were then hand-drawn using crayon to create hydrophobic barriers and then punched to create a pattern for the sensor zone (Mavaei et al. 2021).

Detection of mercury ions was done by observing the color change visually unaided. When the AgNPs solution is exposed to mercury ions, the solution turns from brown into colorless. Concentration-dependent gradient color change enables quantitative analysis of mercury using silver nanoparticles. In the research conducted by Mavaei et al., a smartphone application was used as a portable detector for the visual and quantitative analysis. The HSV (hue saturation value) assessment system was used for the detection in the application. The constructed paper-based sensor was proven to be useful for the analysis of Hg2+ pollutants in diverse real water samples with a limit of detection of $0.30 \times 10{-}6$ M (Mavaei et al. 2021). Research by Sulistyarti et al. also showed that linear measurement of Hg2+ was successfully performed in the range of 0–15 mg/L, with a limit of detection and limit of quantification of 0.039 and 0.130 mg/L, respectively (Sulistyarti et al. 2023).

UV-Visible spectroscopy was used to observe the shift in strength of the surface plasmon resonance (SPR) band. It was discovered that when Hg2+ salt solutions were introduced, the color of the AgNPs solution changed from brownish to transparent, and the SPR frequency for Hg2+ vanished completely. The addition of 20–160 μM Hg2+ to the AgNPs solution allowed for quantitative detection of the mercury ion. Patra S et al. also use green tea extract-mediated AgNPs to produce monodispersed AgNPs. AgNPs were found to interact with Hg2+ ions, forming a Hg − Ag alloy (amalgam), resulting in a color change from brown to no color in the sample. The sensing system has a detection limit of 0.01 mg/L and a quantification limit of 0.04 mg/L, as proven by UV-vis spectroscopy (Patra et al. 2023).

Another approach for green production of nanoparticles was from Suhaidi N et al., who synthesized gold nanoparticles containing the bromelain enzyme (brn-AuNPs) from *Ananas comosus* (Suhaidi et al. 2023). The binding of bromelain to AuNPs (brn-AuNPs) improves enzyme stability and increases AuNP sensitivity (Swaroopanand et al. 2015). The interaction of the brn-AuNPs complex with Hg2+ results in visible aggregation of AuNPs from red to blue. The developed gold nanoparticles successfully detected mercury in water samples with a low colorimetric detection concentration (LOD) of 0.011 ppm, enabling digital image processing.

The recent colorimetry analysis of mercury was conducted by Saputri et al., which used paper immobilized by gold nanoparticles (AuNPs) conjugated with cyanuric acid (CA). The CA-AuNPs filter paper sensor's reaction to exposure to Hg2+ is seen in the obvious color shift from pink to bluish purple. The detection limit is 0.05 μM, and it can be visually viewed with the unaided eye and/or ImageJ software. After testing with various metal ions, the colorimetric response of the sensor was also selective towards Hg2+ (Saputri et al. 2023).

Spectrophotometric analysis

The spectrophotometric approach is extensively used to identify analytes in samples by measuring the sample's absorbance, which indicates the analyte's concentration (Yuniati and Rifai 2019). Spectrophotometric analysis is favored and widely used for the analysis of mercury by researchers around the world. Earlier research typically used UV-Vis spectrophotometry, but in recent years the trend has shifted to methods such as atomic absorption spectrophotometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled mass spectrometry (ICP-MS). Mercury analysis using spectrophotometry usually starts with sample preparation, particularly acid digestion to remove the organic matter in the matrices. Therefore, the use of a direct mercury analyzer has also been explored.

The UV-Vis spectrophotometric method for mercury analysis is favorable because of the low-cost and easily accessible instrument. However, the analysis usually requires a reagent to form a complex with mercury that can be detected in the UV/Vis region (Simião et al. 2022). Commonly used reagents are 6-hydroxy-3-(2-oxoindolin-3-ylideneamino)-2-thioxo-2H-1,3-thiazin-4(3H)-one (Hamza et al. 2010), rhodamin B (Loo et al. 2012), and 2-acetylpyridine thiosemicarbazone (APT) (Babu and Reddy 2012). Hussain A et al. used Michler's thioketone reagent (ligand), a compound with the chemical name [4,4'-Bis(dimethylaminothiobenzophenone)] (C17H20N2S). This reagent formed a dark blue complex with mercury ions at pH 7. The spectrophotometric method is sensitive, with a detection limit of 5.235 × 10-7 M (Hussain et al. 2019). Meanwhile, Alzahrani et al. use Schiff base ligand 2-((5-(2-hydroxy3-methoxybenzylideneamino)-2H-1,2,4-triazole-3-ylimino) methyl)-6-methoxyphenol (HMBT) to produce a complex with mercury in a 2:1 molar ratio (Alzahrani et al. 2023). The HMBT ligand spectra showed a maximum absorption peak at 388 nm, whereas the Hg complex exhibited an absorption maximum at 475 nm. Analysis using HMBT follows Beer's law in the concentration range of 0.1–6 mg/mL of Hg, with (LOD) 0.016 mg/L and (LOQ) 0.051 mg/L (Alzahrani et al. 2023).

The cold vapor-atomic absorption spectrometry (CV-AAS) approach was used by Koesmawati et al. for total mercury analysis in fish samples, using sodium borohydride as a reducing agent. A commonly used method by the US Environmental Protection Agency (US EPA) uses stannous chloride (SnCl2) as a reducing agent. This study found that 0.3% sodium borohydride effectively reduced Hg, and the method was successfully applied in oven-dried and freeze-dried fish meat samples, despite its susceptibility to dissolved oxygen. However, further studies should be conducted to examine the effect of different concentrations of sodium borohydride on the efficacy of the reduction of mercury (Koesmawati et al. 2023).

In addition to CV-AAS, the cold vapor-atomic fluorescence spectrometer (CV-AFS) method is also used in mercury analysis. Astolfi M et al. compared CV-AFS and an advanced mercury analyzer (AMA) to measure total Hg in hair samples (Astolfi et al. 2019). AMA is based on AAS detection and requires no sample preparation, whereas CV-AFS measures atomic fluorescence intensity following acid digestion. Studies carried out using 5 and 20 mg samples of human hair showed that the limits of detection were 0.0004 and 0.004 mg/kg for AMA and CV-AFS, respectively. Ultimately, AMA is recommended over CV-AFS for hair analysis as it simplifies sample handling, reduces contamination risk, requires fewer samples, and enables more cost-effective analyses (Table 2).

Based on the study conducted by Looney et al., a comparison was made between direct mercury analyzer (DMA), CV-AAS, and inductively coupled plasma mass spectroscopy (ICP-MS) to determine mercury in radioactive waste samples (Looney et al. 2022). As with AMA, DMA eliminates the sample preparation process, typically necessary for CV-AAS and ICP-MS analyses, allowing immediate measurement of total mercury by volatilizing the sample and catalytically reducing all mercury atoms. Comparable results were obtained during triplicate total mercury analysis of radioactive waste, which produced average results of 54.1 mg/L (as Hg) with CV-AAS, 55.1 mg/L with ICP-MS, and 56.0 mg/L with DMA.

Electrochemical analysis

One alternative to conventional methods in heavy metal analysis is to use electrochemical sensors. The electrochemical technique is known for its simplicity, rapid response, affordable cost, and portability, as well as good sensitivity for heavy metals analysis (Hwang et al. 2021). Electrochemical analysis is an effective method for determining mercury in various types of samples, with the most common application being water samples. In the case of water samples, typically no sample preparation is necessary. However, for the analysis of mercury in solid or more complex liquid samples, sample preparation is essential. This typically involves extracting mercury from

Table 2. Spectrophotometric technique for the determination of mercury in the sample.

No	Sample	Reagent	Analytical instrument used	Extraction Method	Linearity range	LOD	Ref
1	Water	Schiff base ligand	UV-Visible spectrophotometer	Direct measurement	0.1–6 µg mL- 1	0.016 mg/L	(Alzahrani et al. 2023)
2	Fish	$NaBH_4$ as the reductor	CV-AAS	Acid digestion	–	–	(Koesmawati et al. 2023)
3	Human hair	–	CV-AFS	Acid Digestion	0.01–1.5 µg L^{-1}	0.004 mg/kg	(Astolfi et al. 2019)
4	Human hair	–	AMA using AAS	Does not require sample preparation	0.1–4 ng	0.0004 mg kg^{-1}	(Astolfi et al. 2019)
5	Radioactive waste	–	DMA	Hot vapor atomic absorbance (AA)	4 µg/L (0.4 ng)	4 to 1000 µg/L	(Looney et al. 2022)
6	Radioactive waste	Bromine monochloride and permanganate/ persulfate oxidation	CV-AAS	Acid digestion	–	–	(Looney et al. 2022)
7	Radioactive waste	–	ICP-MS	Acid digestion	–	–	(Looney et al. 2022)

*CV-AAS: cold vapor-atomic absorption spectrometry; CV-AFS: cold vapor-atomic fluorescence spectrometry; DMA: direct mercury analyzer; ICP-MS: inductively coupled plasma mass spectrometry; AMA: advanced mercury analyzer.

the samples and diluting the extract in water prior to analysis. A study by Raril and Manjunatha demonstrated the use of cyclic voltammetry for mercury analysis in human blood serum. The serum was obtained by centrifuging human blood samples, which were then dissolved in PBS and analyzed using the polyglycine-modified graphene paste electrode (PGMGPE) (Raril and Manjunatha 2020). Similarly, Boopathy et al. conducted voltammetric analysis on both human blood serum and fish extract. While the serum samples are directly analyzed using differential pulse voltammetry (DPV), the fish samples were first chopped into small pieces, soaked in pH 7.0 solution, stirred, and filtered before analysis (Boopathy et al. 2019).

In analysis using electrochemical sensors, the role of electrodes is crucial in determining analytical performance; therefore, the properties and type of electrodes used must be considered (Bohari et al. 2020). Commonly used electrodes for target molecule detection by electrochemistry are glass carbon electrodes (GCEs) that are modified to increase the sensitivity to Hg2+ (Singh et al. 2020). The modification of GCEs can be done with nanocomposites (Tian et al. 2020), metal complexes (Sengupta et al. 2021), or conductive and molecularly imprinted polymers (Pardeshi and Dhodapkar 2022; Mirzaei Karazan and Roushani 2023).

Biomaterials such as enzymes, peptides, nucleic acids, and cells are also a popular candidate for electrode modification. Several attempts have been conducted by researchers to establish biomaterial-modified electrodes. Ri Wang et al. performed a study using GCE coated in chitosan/graphene oxide/molybdenum disulfide/Au nanoparticles (chitosan/GO/MoS2/AuNPs) and a thiolated DNA probe of the thymine-Hg2+-thymine base pair for the analysis of Hg2+ in water samples (WANG et al. 2022). Meanwhile, Akbari Hasanjani and Zarei used DNA immobilized onto poly-L-methionine-gold nanoparticles/pencil graphite electrode (DNA/PMET-AuNPs/PGE) (Akbari Hasanjani and Zarei 2019). Testing was carried out on the developed electrode using Differential Pulse Stripping Voltammetry (DPSV) or Square Wave Anodic Stripping Voltammetry (SWASV). For the sensor developed by Wang, the study result showed good linearity from 0.01 μg/L to 4 μg/L with a very low limit of detection (5.8 ng/L), although the recovery still needs improvement.

A study conducted by Meng et al. used single-stranded DNA so that folding into a double-stranded structure occurs in the presence of Hg2+ (Meng et al. 2024). Based on this research, the accuracy of the sensor is impaired due to marine biofouling when tested on seawater. Therefore, antifouling materials such as Ag and Au used to be bactericidal, and zwitterionic peptide design as an antifouling layer is used as well as using PEDOT as a conductive layer to improve signal transmission.

Besides biomaterials, studies were also carried out to develop an electrode that was modified using a conductive polymer. Khodaei and Zanganeh developed a GCE, which was modified usinga conductive polymer called polyaniline (PA), which was then enhanced by doping it with sulfosalicylate (SA) and creating ion-discrimination positions by patterning the PA/SA coating towards mercury (II) ions (Khodaei and Zanganeh 2023). DPASV testing carried out on the electrodes led to a sensitive, selective, and applicable sensor to a river water sample, which was proven by the increase in the recovery before and after the patterning (from 54.8%–85% to 91.8%–97.4%).

In addition to conductive polymers, researchers have established that metal-organic frameworks (MOFs) can also be applied to detect Hg(II) due to their excellent binding ability. The optimized MOFs used in the research conducted by Yang et al. were prepared by combining Zr(IV) and 2,5-dimercaptoterephthalic acid (Zr-DMBD MOFs) and applying it to 3D kenaf stem-derived carbon (3D-KSC) or macroporous carbon electrode (Yang et al. 2020a). The results showed that Zr-DMBD MOFs/3D-KSC electrodes have better performance compared to Zr-DMBD MOFs/GCE. Pandiyan also developed MOF with samarium, using a solvothermal process to synthesize Sm-BTC (Samarium-Benzene tricarboxylic acid), which was then used to modify GCE (Pandiyan et al. 2024). The developed electrode showed good sensitivity and selectivity results when tested by CV and square wave voltammetry (SWV), with better detection limits than Zr-DMBD MOFs/3D-KSC.

Innovations continue to be made by Guessan et al. by modifying the carbon paste electrode (CPE) with *Moringa oleifera* seed powder, which is a flocculant-adsorbent used to treat water (Guessan et al. 2024). *Moringa oleifera* seed powder is a flocculant-adsorbent with amorphous and porous properties, which can facilitate the ion adsorption process. The comparison of the detection power of CPE modified with *Moringa oleifera* (CPEM-MO) with unmodified CPE increased more than 7-fold and had a limit of detection of up to 0.4 nM.

Furthermore, Xiaoyu Wang et al. came up with an electrochemical sensor using a rare metal, terbium vanadate (TbVO4) nanowire-based electrochemical sensor (Wang et al. 2024). In optimal circumstances, the linear range for detecting Hg2+ is 0.01–100 μM. A similar detection limit has also been found with the modified tungsten carbide nanosheets electrode, which is 0.18 nM for Hg2+ detection (Boopathy et al. 2019). Both researches show a good selectivity since the voltammetry peaks are low for the interference species.

Electrochemical methods also allow for the detection of multiple heavy metals simultaneously. For example, Raril and Manjunatha conducted simultaneous detection of Hg(II) and Pb(II) in water and biological samples using a polyglycine-modified graphene paste electrode. The method showed sensitive results to both heavy metals and was applicable to real samples such as groundwater and blood samples with recoveries of 79.92% to 101% for Hg(II) and 86.63% to 101.2% for Pb(II) (Raril and Manjunatha 2020). Bernalte et al. also used screen-printed electrode probes for simultaneous metal detection on Amazon River water on-site (Bernalte et al. 2020). In the study, the quantification of Pb2+, Cu2+, and Hg2+ metals can be done with the presence of three peaks, which occur constantly with a linearity range of 5 to 300 μg L-1 (Table 3).

Table 3. Electrochemical analysis for the determination of mercury in various samples.

No	Sample	Analytical Technique	Electrode	LOD	Linearity range	Sensitivity	Ref
1.	Tap Water	DPSV	Chitosan/GO/MoS_2/AuNPs/DNA/GCE	5.8 ng/L	0.01 μg/L – 4 μg/L	–	(WANG et al. 2022)
2.	Sea Water	SWASV	DNA/PMET-AuNPs/PGE	0.004 aM	0.1 aM - 0.1 nM	–	(Akbari Hasanjanand Zarei 2019)
3	Sea Water	DPV	apt-pep-Au/Ag/PEDO T/GCE	2.30 pM	0.01 nM–100 nM	–	(Meng et al. 2024)
4.	River Water	DPASV	PA/SA/GCE	1.0 × 10–10 M	1.0 × 10–9– 1.0 × 10–4 M	–	(Khodaei and Zanganeh 2023)
5.	Waste Water	SWASV	Zr-DMBD MOFs/3D-KSC	0.05 µM	0.25 µM–3.5 µM	324.58 µA/µM/cm	(Yang et al. 2020b)
6.	Water	SWV	Sm-BTC/GCE	10 nM	0.01 µM–50 µM	91.760 µA/µM/cm	(Pandiyan et al. 2024)
7.	Waste Water	ASV	MO/CPE	0.4 nM	0.002 µM–0.009 µM	–	(Guessan et al. 2024)
8.	Water	SWV	TbVO4/GCE	0.18 nM	0.01 µM–100 µM	–	(Wang et al. 2024)
9.	Human Blood Serum	DPV	WC NSs/SPCE	0.19 nM	0.002–655 µM. L–1	8.87 µA nM–1 L–1 cm–2	(Boopath et al. 2019)
10.	Water and Human Blood Serum	CV	PG/GPE	6.6 µM	1.5 × 10–4 to 1.5 × 10- 3 M	–	(Raril and Manjunatha 2020)
11.	River Water	SWASV	Au SPEs	1.3 µg L–1	5–300 µg/L	–	(Bernalte et al. 2020)

*ASV: anodic stripping voltammetry; CV: cyclic voltammetry; DPASV: differential pulse anodic stripping voltammetry; DPV: differential pulse voltammetry; DPSV: differential pulse stripping voltammetry; SWASV: square wave anodic stripping voltammetry; SWV: square wave voltammetry.

Chromatographic analysis

Chromatographic analysis separates sample components based on subtle differences in solubility, resolution, adsorption, and desorption between the stationary and mobile phases (Wang et al. 2021). Chromatography is ideal for the separation and detection of mercury in complex samples, such as food and biological matrices. When analyzing biological samples such as human hair, techniques that combine liquid or gas chromatography separation with highly sensitive detectors, such as electron capture detector atomic fluorescence spectroscopy and ICP-MS, are preferred over non-chromatographic methods. This technique is faster, more automatic, and generally capable of detecting organic mercury species down to the microgram per kilogram (µg/kg) level (Spanu et al. 2024). Unlike gas chromatography, liquid chromatography doesn't need mercury speciation to be turned into volatile compounds before separating them, making it a good choice for analysis. While many methods exist for detecting mercury speciation in biological samples using HPLC-ICP-MS, they often require a lot of solvents and create a high amount of waste containing MeHg (Suratno et al. 2023). Preceding the chromatographic analysis, extraction of mercury can be done with various methods such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), and microwave-assisted extraction.

Common extraction methods for mercury utilize solvents such as methanesulfonic acid, mercaptoethanol, L-cysteine, pepsin, and tetramethylammonium hydroxide (TMAH). Acids such as nitric and hydrochloric acid and hydrogen peroxide also can be used in the extraction process. Digestion of matrices utilized several techniques to accelerate the extraction process, such as the use of microwave heating, a closed vessel using a conventional oven, or the cold finger method, which uses a glass tube placed over the digester tube to create a reflux system that minimizes loss of analyte by evaporation (Ferreira et al. 2015).

An example of chromatographic mercury analysis uses gas chromatography-mass spectrometry (GC-MS) with derivatization using tetraaryl borate salt. A study performed by Lyu et al. successfully quantifies MeHg and EtHg bromide complexes in blood samples. The organic mercury was extracted using liquid-liquid extraction (LLE), first with a cysteine/alkaline solution, methyl isobutyl ketone (MIBK), and then with a toluene/copper chloride solution before undergoing derivatization using tetraaryl borate salt. The developed method showed a limit of detection for MeHg and EtHg bromide complexes of 0.12 ng/mL and 0.14 ng/mL, respectively (Lyu et al. 2023).

HPLC-ICP-MS is highly preferred because it can analyze multiple elements all at once in a single run. Its seamless integration of separation and detection systems, along with its detector's adaptability and sensitivity, makes it a versatile choice (Favilli et al. 2022). Bi et al. conducted a study to synthesize new magnetic material that is able to extract various types of mercury from water and fish samples (Bi et al. 2021). Aside from HPLC, frontal chromatography can also be combined with ICP-MS (FC-ICP-MS), which uses a low-pressure column. This simplification not only reduces instrument complexity and cost but also facilitates quick and selective detection of methylmercury in biological samples. The process was quick, which took 15 minutes for sample preparation and 5 minutes for analysis. The sample preparation uses thiourea as a sulfur-based complexant for Hg in acidic pH. The method was able to detect trace amounts of organomercury in small hair samples, even as little as 5.5 µg/kg (Spanu et al. 2024).

Due to the low concentration of mercury found in biological samples, preconcentration methods are usually

required to detect them before chromatographic analysis. Over the years, many methods have been developed to extract mercury species and their various elements before chromatography analysis. One popular method is solid phase extraction (SPE), which is easy to use with liquid chromatography instruments, particularly with HPLC coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). It is well-tested and preferred for its high recovery, easy automation, and flexibility. The extraction techniques used can influence the choice of HPLC phase, which is important for efficiency. The SPE column used for mercury extraction from samples includes graphene oxide and carbon nanotube, with thiourea and L-cysteine as the eluting solvent. For the HPLC-ICP-MS analysis, reverse-phase is usually used in conjunction with mobile phases such as L-cysteine and 2-mercaptoethanol, combined with methanol and ammonium acetate to aid detection sensitivity and retention time.

For multielement analysis, conditions vary based on the element studied, and methods must be adapted (Favilli et al. 2022).

A more recent approach gaining attention in recent years is dispersive liquid–liquid microextraction (DLLME). It involves dispersing an immiscible extractant phase into the sample using a dispersing agent, typically an organic solvent. DLLME offers several advantages, such as rapid extraction time, high enrichment factors, ease of use, low cost, and high extraction efficiency. However, two main disadvantages arise when using organic solvents as dispersing agents. Firstly, the partition coefficient of the analyte in the extractant phase decreases, and secondly, an organic solvent usually is not environmentally friendly (Ripoll et al. 2023).

Mercury extraction can also be achieved using natural deep eutectic solvents (NADES). a subclass of DES derived from natural sources. NADES offer advantages such as low toxicity, minimal vapor pressure, high thermal stability, ease of synthesis at ambient conditions, high purity, and cost-effectiveness. Additionally, DES is recognized as a cost-effective replacement for ionic liquids. The method developed by Ripoll et al. involves the use of a specific NADES (decanoic acid:DL-menthol 1:2) to concentrate mercury species (MeHg+, EtHg+, PheHg+, Hg2+) using DLLME before LC-UV-Vis analysis. Prior to extraction, 100 mL of dithizone solution was added to form a dithizonate-mercury complex. The LOD and LOQ of the method are 0.9 mg/L and 3 mg/L for MeHg+, EtHg+, PheHg+; and 3 mg/L and 10 mg/L for Hg2+. The environmental impact of this method is measured using the AGREEprep metric, which shows that this method is an environmentally friendly analysis method (Ripoll et al. 2023) (Table 4).

Miscellaneous

Current advances in mercury analysis were not limited to the common colorimetric, electrochemical, spectrophotometric, and chromatographic methods. Techniques were developed to enhance the effectiveness of mercury extraction and speciation before further analysis, such as using ion-imprinted polymers, resins, and nanosheets to aid in the sample pretreatment. A recent study also showed that silver nanoparticles (AgNPs), commonly used in the colorimetric determination of mercury, can also be used in sample preconcentration in a modified membrane.

In a study conducted by Low et al., an ion-imprinted polymer (IIP) was developed from fluorescein and 2-aminophenol functionalized methacrylic acid monomer. This IIP solution was then coated onto the quartz crystal microbalance (QCM) and assembled into the

Table 4. Chromatographic analysis used for the determination of mercury in various samples.

No	Sample	Analytical instrument used	Extraction Method	Linearity range	LOD	Ref
1	Human hair	FC-ICP-MS	Microwave-assisted digestion	10 µg/kg	5.5 µg/kg	(Spanu et al. 2024)
2	Fish, oyster, and water	LC-ICP-MS	Magnetic solid-phase extraction	3.2–1000, 0.55–1000 and 1.57– 1000 ng L^{-1} for IHg, MeHg, and EtHg	0.96 0.17 0.47 ng/L for IHg, MeHg, and EtHg	(Bi et al. 2021)
3	Water, air, biota, and sediment	LC-ICP-MS	Solid-phase extraction (SPE)	–	0.001– 0.007 ng L	(Favilli et al. 2022)
4	Shark meat	UHPLC-ICP-MS	Sonication-assisted extraction	–	0.86 pg/L	(Suratno et al. 2023)
5	Blood	GC-NCI-MS	Used MIBK (methyl isobutyl ketone)	0.02 ng/mL to 20 ng/mL	0.12 ng/mL and 0.14 ng/mL for MeHgBr and EtHgBr	(Lyu et al. 2023)
6	Fish, rice, and soil.	LC-ICP-MS	Microwave-assisted extraction	–	11.2, 11.5 12.3 µg kg^{-1} in rice, fish, and soil	(Anil et al. 2024)
7	Water	LC-ICP-MS	Magnetic solid-phase extraction	0.26–1000 ng/L	0.08–0.90 ng/L	(Zhang et al. 2024)
8	Water	LC-UV-Vis	Dispersive liquid-liquid microextraction (DLLME)	12–200 µg L−1	3 µg L−1	(Ripoll et al. 2023)

*FC-ICP-MS: frontal chromatography-inductively coupled plasma mass spectrometry; HPLC-ICP-MS: high-performance liquid chromatography-inductively coupled plasma mass spectrometry; GC-NCI-MS: gas chromatography-negative chemical ionization mass spectrometry; UHPLC-ICP-MS: ultra-high-performance liquid chromatography-inductively coupled plasma mass spectrometry.

QCM-D analyzer apparatus. The IIP-QCM method was found to have a high selectivity towards Hg2+, with LOD and LOQ values of 14.17 and 42.94 ppb. The method has also been successfully applied for mercury detection in tap water and palm oil wastewater samples without further extraction processes (Low et al. 2024).

Detection of mercury can also be enhanced using a new resin called CYXAD (CYPHOS 101 modified Amberlite XAD), which effectively separates different forms of mercury, including total mercury, inorganic mercury, and methylmercury in fish samples. For total mercury testing, a simple mixture of nitric acid and hydrogen peroxide helps extract mercury from the sample. For further speciation, solid-phase extraction is carried out using a cartridge containing CYXAD resin, with the assistance of hydrochloric acid and hydrogen peroxide. Determination of mercury from the extracts was then conducted using anodic stripping voltammetry (ASV). These pieces of equipment, along with their supporting appliances, are easily portable, allowing for efficient outdoor mercury testing in fish samples. This eliminates the need for lengthy extraction processes and hazardous solvents, making the testing procedure faster and more environmentally friendly (Inaudi et al. 2022).

A study by Ahmad et al. (2022) highlights the utilization of two-dimensional molybdenum disulfide (MoS2) nanosheets to enhance DSPME (dispersive solid-phase microextraction) of trace Hg(II). The MoS2 nanosheets, prepared through a hydrothermal method without the use of intercalating agents, displayed expanded interlayer spacing. The primary mechanism for Hg(II) adsorption likely involves the formation of strong Hg(II)-S complexes, in addition to electrostatic interactions. Extraction is performed by dropping the nanosheets onto the sample solution (pH adjusted to 6). The mixture is then sonicated and centrifuged, followed by elution of the sorbed Hg(II) using nitric acid for further analysis using ICP-OES. These nanosheets enable quantitative analysis of trace Hg(II) at concentrations as low as ng/mL without interference from common co-existing ions. The effectiveness of the proposed DSPME method for Hg(II) extraction has been validated through the analysis of real water and industrial wastewater samples (Ahmad et al. 2022).

To ensure safety, it is important to detect even small amounts of mercury, which is known to have harmful effects. A new method by Fadillah et al. designed a membrane using special filter paper modified with silver nanoparticles (AgNPs), green tea extract, and 3-amino propyl trimethoxy silane (APTMS) to preconcentrate mercury ions from cosmetic samples. Green tea extract was used as a reducing agent for AgNPs, and APTMS was added to enhance adsorption effectiveness toward Hg2+ ions. Cosmetic samples were prepared by dissolving them in HNO3 followed by heating. The solution was then filtered using the AgNPs-APTMS modified filter paper and measured using voltammetry. The result of validation showed that the method is highly sensitive, and LOD and LOQ were 0.0483 mM and 0.1611 mM, respectively (Fadillah et al. 2024) (Table 5).

Table 5. Miscellaneous analysis used for the determination of mercury in various samples.

No	Sample	Analytical method used	Extraction Method	Linearity range	LOD	Ref
1	Wastewater	Ion-imprinted polymer (IIP) combined with a quartz crystal microbalance (QCM)	–	42.94 ppb to 2 ppm	14.17 ppb	(Low et al. 2024)
2	Tuna Fish	Home-made columns called CYXAD. Determined by anodic stripping voltammetry (ASV), using a solid gold electrode (SGE)	Solid-Phase Extraction	1.71 μA/μgL−1 and 1.68 μA/μgL−1 for HgIN CH3Hg	0.40 μg L−1 for HgIN and 0.50 μg L−1 for CH3Hg	(Inaudi et al. 2022)
3	Water	DSPME, followed by ICP-OES analysis	Dispersive solid-phase microextraction (DSPME)	–	0.01 ng/mL	(Ahmad et al. 2022)
4	Cosmetic	Membrane using voltammetry analysis	Solid-phase extraction (SPE)	–	0.0483 mM	(Fadillah et al. 2024)

Conclusion

Mercury is a heavy metal that requires careful monitoring due to its impact on the environment and human health. Therefore, reliable analytical methods are essential for detecting and determining mercury in both environmental and biological samples. Automated analytical instruments like AAS, LC, and ICP, which utilize various detectors as well as direct detection techniques, are some of the commonly used techniques. These instruments offer excellent sensitivity, accuracy, and precision, as well as a wide application in different kinds of samples. However, a certain sample preparation method is typically required before performing the analysis. Simpler approaches, such as rapid colorimetric testing, can be used for on-site analysis but tend to be semi-quantitative. Ongoing investigations to enhance the sample preparation procedures and analytical techniques are needed in order to provide a more effective, affordable, sensitive, and environmentally friendly mercury analysis.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.

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