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A rapid ultrasound-assisted thiourea extraction method for the determination of inorganic and methyl mercury in biological and environmental samples by CVAAS

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Abstract

A rapid ultrasound-assisted extraction procedure for the determination of total mercury, inorganic and methyl mercury (MM) in various environmental matrices (animal tissues, samples of plant origin and coal fly ash) has been developed. The mercury contents were estimated by cold vapour atomic absorption spectrometry (CVAAS). Inorganic mercury (IM) was determined using $SnCl_2$ as reducing agent whereas total mercury was determined after oxidation of methyl mercury through UV irradiation. Operational parameters such as extractant composition (HNO_3 and thiourea), sonication time and sonication amplitude found to be different for different matrices and were optimized using IAEA-350 (Fish homogenate), IM and MM loaded moss and NIST-1633b (Coal fly ash) to get quantitative extraction of total mercury. The method was further validated through the analysis of additional certified reference materials (RM): NRCC-DORM2 (Dogfish muscle), NRCC-DOLT1 (Dogfish liver) and IAEA-336 (Lichen). Quantitative recovery of total Hg was achieved using mixtures of 5% HNO_3 and 0.02% thiourea, 10% HNO_3 and 0.02% thiourea, 20% HNO_3 and 0.2% thiourea for fish tissues, plant matrices and coal fly ash samples, respectively. The results obtained were in close agreement with certified values with an overall precision in the range of 5–15%. The proposed ultrasound-assisted extraction procedure significantly reduces the time required for sample treatment for the extraction of Hg species. The extracted mercury species are very stable even after 24 h of sonication. Closed microwave digestion was also used for comparison purposes. The proposed method was applied for the determination of Hg in field samples of lichens, mosses, coal fly ash and coal samples

Keywords: Ultrasound-assisted extraction; Mercury; Fish homogenate; Lichens; Moss; Coal fly ash; CVAAS

1. Introduction

Mercury is a global pollutant and is identified as a highly toxic element because of its accumulative and persistent character in the environment [1]. Various mercury species differ greatly in their bio-physico-chemical properties such as toxicity, solubility, and rate of bioaccumulation by organisms, etc. [2,3]. The most toxic are monomethyl mercury compounds, which represent a health risk, particularly to the foetal neurosystem because of its enhanced toxicity, lipophilicity, bioaccumulation and volatility [4]. Inorganic mercury (IM) is methylated by sulfate reducing or methanogenic bacteria and transmethylation reactions with organometallics [5] and becomes highly toxic methyl mercury (MM).

Mercury and its compounds are present as trace contaminants in various biological and environmental samples such as animal tissues, plant matrices and coal fly ash, as a result of both natural and anthropogenic activities [6]. The highly toxic and fat-soluble mercury species tend to accumulate in fish tissue, from where they can re-enter the human food chain. A sample of plant origin particularly lichens and mosses are known for their ability to accumulate various toxic elements including mercury [7,8]. Measurements of Hg in lichens and mosses are of particular importance for spatial monitoring of

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Hg in air around pollution sources. The natural reserves of mineral coal are an important non-renewable energy source in our planet. It is very important to know the concentration of toxic elements particularly mercury in coal and coal fly ash due to environmental problems associated with the use of coal, particularly in coal fired power plants [9].

Inorganic mercury (Hg^{2+}) and methyl mercury (CH_3Hg^+) are the two major species generally found in various biological samples [10]. Hence, the analysis of samples only for total mercury is therefore no longer completely acceptable because it provides only partial information about their impact on human health and the environment. As a consequence, considerable effort and progress have been made in the development of techniques, which are capable of separating and identifying the various mercury species [11,12]. During the last two decades, many laboratories have carried out intensive investigations and as a forum for the presentation of the results, the International Conference series on Mercury as a Global Pollutant (ICMGP) was initiated in 1990 and the most recent one was held in Slovenia during June–July 2004.

The extraction of mercury species from the sample matrix is recognized as one of the most important steps. The main concerns in extraction should be the volatility, loss of mercury during elevated temperature digestion procedures, interspecies conversion, sample contamination and the problem of using a large amount of reagents during pre-treatment which gives rise to increased blank values and higher detection limits. The pre-treatment method should be able to solubilize the organic Hg species from the sample of interest without breaking the C–Hg bond. A variety of analytical methods for the extraction of mercury species in biological samples have been published and reviewed [6,12–15].

The first methods used to extract mercury compounds from fish were developed in the 1960s [16] but the protocol was developed in 1966 by Westoo [17] was the first that focused on mercury speciation rather than total levels. Other methodologies such as acid leaching [18,19], alkaline leaching with either strong or weak bases [20–23] (e.g., methanolic-potassium hydroxide or tetramethyl ammonium hydroxide (TMAH), aqueous distillation [21–24], supercritical fluid extraction [25] and microwave-assisted extraction [20,26], Grignard reagents [27,28] have been developed for the extraction of mercury species as alternatives to the Westoo procedure. Tutschku et al. [29] have established and compared different methodologies based on acid microwave digestion, steam distillation, acid extraction and alkaline dissolution for the determination of certified concentration of MM in SRM 1566b Oyster tissue and SRM 2977 Mussel tissue.

The most common method used in mercury speciation studies is gas chromatography (GC) followed by electron capture detection (ECD) [26,30]. The low sensitivity associated with this detector for organomercury species is improved by replacing the ECD with atomic absorption spectrometry (AAS) [20], microwave induced plasma atomic emission spectrometry (MIP-AES) [25], atomic fluorescence spectrometry (AFS) [31] or inductively coupled plasma mass spectrometry (ICP-MS) [14,15,32]. The use of high performance liquid chromatography (HPLC) to separate mercury species has been reviewed [33]. HPLC has also been coupled to several detection techniques such as AAS [34], AFS [35,36] or ICP-MS [37,38].

Despite excellent sensitivity and selectivity, most of the above-mentioned techniques suffer from disadvantages such as laboriousness of the procedures, use of concentrated acids, lack of acceptable efficiency, time consuming procedures, contamination, loss of mercury, etc. [6]. A successful leaching (or extraction) procedure prior to speciation analysis requires the preservation of all original compounds, such as organometallic compounds present in the samples. Although the microwave-assisted extraction shortens the sample preparation time, degradation of MM to IM and evaporation losses have been observed after acid leaching in microwave field [20].

In this context, the use of an ultrasonic probe can be an excellent alternative to minimize the disadvantages of conventional extraction procedures in terms of number of analytical steps, time, extraction efficiency and reagent consumption by facilitating and accelerating pre-treatment process of various biological and environmental samples [39]. The efficiency of both microwave [40,41] and ultrasound-assisted extraction [39,42] methods for the sample preparation has been evaluated for various biological and environmental matrices.

A number of authors' emphasized different applications of ultrasound to sample treatment of agricultural, biological and environmental samples for the analysis of various elements and speciation purposes [39,42–45]. A new oxidation method based on room-temperature ultrasonic irradiation (sonolysis) was proposed for conversion of organomercurials in to IM. Ultrasound-assisted extraction has been found to be effective in extracting organic pollutants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls [39]. Rio-Segade and Bendicho have developed an ultrasound-assisted extraction method with 2 and 4 mol l^{-1} HCl for the mercury speciation in fish tissues [45]. The extraction of MM was quantitative using 2 mol l^{-1} HCl, but IM too was leached to an extent of 30% in this step. In our earlier work [46], ultrasound-assisted extraction procedure was used for the fast estimation of major, minor and trace elements (except mercury) in IAEA-336 Lichen and NIST 2976 Mussel tissue. During these studies, it was observed that the mercury extraction was found to be very low (<10%) when HNO_3 alone was used as extractant.

Based on these observations, an ultrasound-assisted extraction procedure has been developed for the quantitative extraction of IM and MM from various bio-environmental matrices such as fish homogenate, lichens, mosses, coal fly ash, using a mixture of thiourea and HNO_3 as the extractant. IM was determined using $SnCl_2$ as reducing agent whereas total mercury was determined after oxidation of MM to IM through UV irradiation followed by its reduction to elemental mercury. The method was validated by the analysis of various certified reference materials (RM): IAEA-350 (Tuna fish),

NRCC-DORM2 (Dogfish muscle), NRCC-DOLT1 (Dogfish liver), IAEA-336 (Lichen) and NIST 1633b (Coal fly ash). Various field samples of lichens, mosses, coal fly ash and coal samples have been successfully analysed for their mercury contents.

2. Experimental

2.1. Instrumentation

Mercury was analyzed by cold vapour atomic absorption spectrometry (CVAAS) using a mercury analyzer (Model MA 5840E, Electronics Corporation of India Ltd., Hyderabad, India). CVAAS is the most widely used technique for Hg determination because of its high sensitivity, absence of spectral interferences, relatively low operational costs, simplicity and speed [47].

2.2. High intensity probe sonicator

A 130 W power and 20 kHz frequency (Cole Parmer instruments, Illinois, USA, Model: CP 130PB-1) high intensity probe sonicator equipped with a Ti probe was used for ultrasound-assisted extraction. The amplitude control of the ultrasonic processor allowed the ultrasonic vibrations at the probe to be set at desired level in the 10–100% range of the nominal power. Polypropylene centrifuge tubes of 50 ml capacity were used for sonication experiments. After sonication, all the extracts were centrifuged at 4000 rpm for about 5 min for the rapid separation of the solid–liquid mixture. A domestic microwave oven (650 W), programmable for the time and microwave power, was used for the total digestion of the sample.

2.3. Reagents and standards

All chemicals were of analytical grade unless otherwise stated. Sub-boiled HCl and HNO_3 were prepared in our laboratory by sub-boiling distillation in quartz stills. Ultra-pure water with >18 MΩ cm resistivity, obtained using a Milli-Q high purity water system, located in class 200 area, was used for dilution of standards, for preparing samples and for final rinsing of the acid cleaned vessels. All containers were soaked in 20% HNO_3 and cleaned thoroughly with high purity water prior to use.

Tin(II) chloride ($SnCl_2$) (5%) used as reducing agent was prepared by dissolving the appropriate amount of $SnCl_2{\cdot}2H_2O$ (Merck, India) in HCl and diluting with water. Sodiumborohydride ($NaBH_4$) (Merck, Darmstadt, Germany) (0.5%) was prepared fresh daily by dissolving the solid in 0.2% NaOH solution. Ten percent HCl was used as carrier. Thiourea (NH_2CSNH_2) (Merck, Darmstadt, Germany), a non-polluting reagent which has been widely used for extracting precious metals [48] and preparation of thiourea-based coordinating resins for binding of mercury as well as eluent for stripping of mercury [49], was used in the extraction studies.

Inorganic mercury (Hg^{2+}) stock standard solution (1000 mg/l) was prepared from mercury chloride (Merck). A methyl mercury (CH_3Hg^+) stock standard solution (100 mg/l, Hg as MM) was prepared from methyl mercury chloride (Merck) by dissolving appropriate amount of the solid in acetone and making up to volume with high purity water. All the stock standard solutions were stored in a refrigerator at 4 °C and protected from light. Working standard solutions were prepared just before use by appropriate dilution of the stock standard solutions.

2.4. Preparation of IM, MM and mixture of both IM and MM loaded moss material under laboratory conditions

Reference materials are available either as marine materials (high MM-Hg) or as terrestrial matrices (low MM-Hg). In most of the RMs either inorganic or methyl mercury is found to be at much higher concentrations relative to the other species. The current status and future needs for mercury reference materials have been summarized by Horvat [50]. To our knowledge no reference material available, which is certified for fairly higher contents of both IM and MM for the analysis of plant samples. In view of this, moss samples loaded with known content of IM (M-IM), MM (M-MM) and mixture of both IM and MM (M-IM–MM) were prepared in the laboratory for use in optimization experiments related to samples of plant origin.

2.4.1. Choice of sorbent for the preparation of Hg loaded moss material

Lichens and mosses, which depend on surface sorption of nutrients, have been widely used in various trace element biomonitoring surveys particularly for mercury [7,8,51,52]. Metal uptake occurs in living or dead organisms and metabolic activity is not needed [53]. Our previous studies on mercury uptake efficiency studies by lichens (*Parmelia sulcata*) and mosses (*Funaria hygrometrica*) have shown that the capacities of lichen and moss for IM were found to be 47 and 52 mg g^{-1}, respectively [54]. In the case of CH_3Hg^+ (in terms of Hg), the capacities were found to be ∼17 and ∼19 mg g^{-1} for lichen and moss, respectively. Moss (*Funaria hygrometrica*) was chosen for preparation of mercury-loaded moss under laboratory conditions due to its wide availability when compared to lichens. Collection of moss samples as well as the preparation of sorbents in the form of powder has been described elsewhere [52].

About 5 g of the powdered moss samples were placed in a 100 ml polypropylene container with a stopper containing 50 ml of high purity water mixed with 25 μg absolute amount of mercury (IM or MM separately) such that the amount of mercury in moss is about 5 μg g^{-1}. The final pH of the solution was adjusted to 5. These were placed on a mechanical shaker for about 1 h to facilitate uniform loading of mercury.

After shaking for 1 h, the mixture was separated by centrifugation (4000 rpm for 5 min) and the supernatant was drained. Then, the sorbent was initially allowed to dry at room temperature. Then, the dried sample was finely ground and then again dried in a conventional heating oven at ∼40 °C to remove the residual moisture. In another set of experiments both IM and MM (12.5 μg each) together were loaded on moss (weight of moss 5 g) using a procedure similar to that described above (M-IM–MM).

2.5. Microwave-assisted procedure for the digestion of the samples for analysis of total mercury

Total mercury concentrations in all the samples were determined after digestion of the samples using closed microwave digestion in a Parr digestion vessel. Accurately weighed amount (100 mg) of IAEA-350, IAEA-336, M-IM, M-MM, M-IM–MM samples were placed separately in PTFE vessels, 2 ml of sub-boiled HNO_3 and 0.5 ml of H_2O_2 were added and then closed. Then, the vessel was put into a Parr digestion vessel and closed. The closed vessels were placed inside a domestic microwave oven (650 W) where they were irradiated for total time of 4 min at maximum power in two 2-min steps using cooling period of about 5 min after first step to avoid an excess of pressure. After cooling to room temperature, the vessels were opened and the sample was diluted to required volume with high purity water. After digestion, the sample digests were analysed for total Hg by CVAAS.

2.6. Ultrasound-assisted extraction procedure

Accurately weighed amount (0.1–0.2 g) of different aliquots of IAEA-350, NIST-1633b, IAEA-336, M-IM, M-MM and M-IM–MM samples were placed in polypropylene centrifuge tubes (50 ml volume) and 5 ml of desired extractant (HNO_3–thiourea) solution was added. Then, the sample–extractant mixture was sonicated at room temperature for predetermined sonication time and sonication amplitude settings. After sonication, the supernatant was separated from the solid phase by centrifugation for about 5 min at 4000 rpm. The known volume of the supernatant was then transferred to another pre-cleaned centrifuge tube. One part of the split samples was analysed for IM by CVAAS using $SnCl_2$ as reducing agent. Other part of the split sample was used for UV irradiation treatment to convert MM to IM and total mercury was analysed by CVAAS using $SnCl_2$/or $NaBH_4$ as reducing agents. Corresponding process blanks were also prepared in the same manner without any sample material. Three aliquots of each sample were used for extraction procedures. With each series of extractions blank was also determined in parallel.

Quantitative extraction of IM and MM from solid samples with probe sonication may not be equally effective for IM and MM under identical conditions, so maximizing the extraction yield requires the process variables to be optimized for each specific matrix [39]. In general, the extraction efficiency is essentially governed by acid concentration, sonication time and sonication amplitude. In view of this, various experiments have been carried out to optimize these variables for quantitative recovery of both IM and MM. Hg loaded moss (representative of samples of plant origin), IAEA-350 (Tuna fish) (representative of animal tissues) and NIST-1633b (Coal fly ash) (representative of coal based samples) were used for optimization experiments.

The reducing reagent solutions, 5% $SnCl_2$ or 1% $NaBH_4$ were used to reduce the mercurial species to elemental mercury. $SnCl_2$ can reduce only Hg^{2+} to elemental mercury but cannot reduce MM, whereas $NaBH_4$ can reduce both Hg^{2+} and CH_3Hg^+ to elemental mercury, albeit with different efficiencies [55].

2.6.1. Optimization of composition of extractant mixture

Among the acids employed as extractants, HNO_3 is reported to have an enhanced performance due to its oxidizing properties. But quantitative extraction of Hg could not be achieved with sonication of the samples up to 10% HNO_3 when employed alone. With the aim of improving extraction efficiency of IM and MM, a mixture of HNO_3 and a complexing agent thiourea or cysteine was used as an extractant in order to avoid use of high concentrated acids. In order to optimize the concentrations of HNO_3 and thiourea, a factorial (two factors, three level) experimental design approach was applied and the recovery of both IM and MM at each level of the treatment was estimated. Based on the results obtained from various preliminary experiments, different compositions of mixture of HNO_3 and thiourea were chosen for different matrices.

The base level was chosen as 5% HNO_3 and 0.02% thiourea for fish homogenate tissues (the upper and lower levels were obtained using a difference of ±2.5% for HNO_3 and ±0.01% for thiourea from the base level), 10% HNO_3 and 0.02% thiourea for moss loaded samples (the upper and lower levels were obtained using a difference of ±5% for HNO_3 and ±0.01% for thiourea from the base level) and 15% HNO_3 and 0.2% thiourea for coal fly ash (the upper and lower levels were obtained using a difference of ±5% for HNO_3 and ±0.1% for thiourea from the base level).

A similar approach was followed for optimization of the concentration of HNO_3 and cysteine using 5% of HNO_3 and 0.5% cysteine as a base line for fish tissues. The upper and lower levels were obtained using a difference of ±2.5% for HNO_3 and ±0.25% for cysteine from the base level. Corresponding extractant solutions were prepared in parallel and employed as blanks. An extractant volume of 5 ml was maintained in all the ultrasound extraction studies.

2.6.2. Optimization of sonication time and amplitude

Since the sonication time, which is the time required for quantitative extraction of analyte of interest, is one of the important parameters influencing ultrasound-assisted extraction [56], sonication time was optimized in order to establish the

best extraction time conditions by keeping ultrasound amplitude (40%), extractant concentration, extractant volume (5 ml) and sample weight (100–200 mg) constant. The sonication amplitude was varied between 20 and 80% whereas extractant concentration, extractant volume (5 ml), sonication time (4 min) and sample weight (100–200 mg) was kept constant.

2.7. UV irradiation as oxidation procedure for methyl mercury

IM can be determined directly in a sample using $SnCl_2$ as reducing agent and total Hg can be determined by prior decomposition of organomercury species into inorganic mercury that can be determined along with original IM. MM is usually determined by difference. Various authors [57,58] have reported about UV irradiation method for the destruction of organic compounds to improve the detection limits. Similar UV irradiation method was used for the oxidation of MM to IM after sonication. An 8-W UV lamp (Philips (India), length 30 cm and diameter 15 mm) was enclosed in a box for eye protection and a PTFE tubing of about 5 m (i.d. 0.5 mm), which was found to be optimum [58] for quantitative conversion (>95%) of MM to IM, pulled over the lamp. Using a peristaltic pump, sample solution was passed through the PTFE coil at an optimized flow rate of 1 ml/min. The sample solution was collected at the out let of the coil and analysed for total mercury using $SnCl_2$ or $NaBH_4$ as reducing agents.

3. Results and discussion

Variables influencing the extraction process were optimized within the intervals shown in Table 1. Since major organomercury species in environmental samples is MM, only recovery studies of IM and MM were carried out throughout this work. IAEA-350, M-IM–MM and NIST-1633b were used for optimization experiments. All the mercury standard solutions, which were prepared in corresponding extractant solution used for quantification of mercury.

3.1. Effect of composition of extractant mixture

Extractant composition was seen to be the most important critical parameter affecting the ultrasound-assisted extraction of mercury species. The propagation of ultrasonic waves is more effective in lower concentrations of acid due to lower viscosity/density of the medium. At higher concentrations of acid, the cavitation process is more difficult to be induced and number of cavitation bubbles per unit volume is reduced [59]. An extractant volume of 5 ml as has been employed by various authors for solid–liquid extraction of metals [45,60] was chosen in the present study so that the required number of replicates could be performed without exhaustion of the sample solution. The most extensively used acid in sonication experiments is HNO_3, although other acids such as HCl, H_2SO_4 or combinations have also been employed [42]. In most of the cases, the typical acid concentration in probe sonication is less than 1 mol l^{-1}.

The first set of factorial experiments was carried out to study the efficiency of the ultrasonic treatment with various compositions of HNO_3 and cysteine. It was found that extractant mixture of 5% HNO_3 and 0.25% cysteine is capable of extracting both the mercury species from fish tissues where as a mixture of 10% HNO_3 and 0.25% cysteine was required for quantitative extraction (>95%) of both IM and MM from plant samples. But cysteine severely interfered in the UV-oxidation process of MM to IM. Hence, further studies were continued only with the mixture of HNO_3 and thiourea as it has no significant effect in the oxidation process of MM (max concentration of thiourea used was 0.02% for biological samples).

Various optimization experiments were carried out with M-IM–MM for the selective extraction of MM. But partial extraction of IM (20–30%) along with MM was noticed with different extractant compositions as has been observed by earlier authors as well [45]. Hence, subsequent optimization experiments were carried out for the quantitative extraction of both IM and MM species (total mercury) in a single step. The results obtained when mixtures with different compositions of HNO_3 and thiourea were used as extractant for the quanti-

Table 1
Experimental conditions for the ultra-sound assisted extraction total Hg from various sample matrices

Variable parameter	Studied interval	Optimum extraction conditions obtained
Sonication amplitude (%)	20–80	40 (Fish tissues and plant samples) 50 (Coal fly ash/coal samples)
Sonication time (min)	1–6	3 (Fish tissues and plant samples) 4 (Coal fly ash/coal samples)
HNO_3 (v/v)% (extractant)	0–7.5 0–15 0–20	5 (Fish tissues) 10 (Plant samples) 20 (Coal fly ash)
Percent of thiourea	0.01–0.05 0.1–0.3	0.02 (Fish tissues and plant samples) 0.2 (Coal fly ash)
Sample amount (g)	0.05–0.5 0.2–0.5	0.05–0.2 (Fish tissues and plant samples) 0.2–0.3 (Coal fly ash)

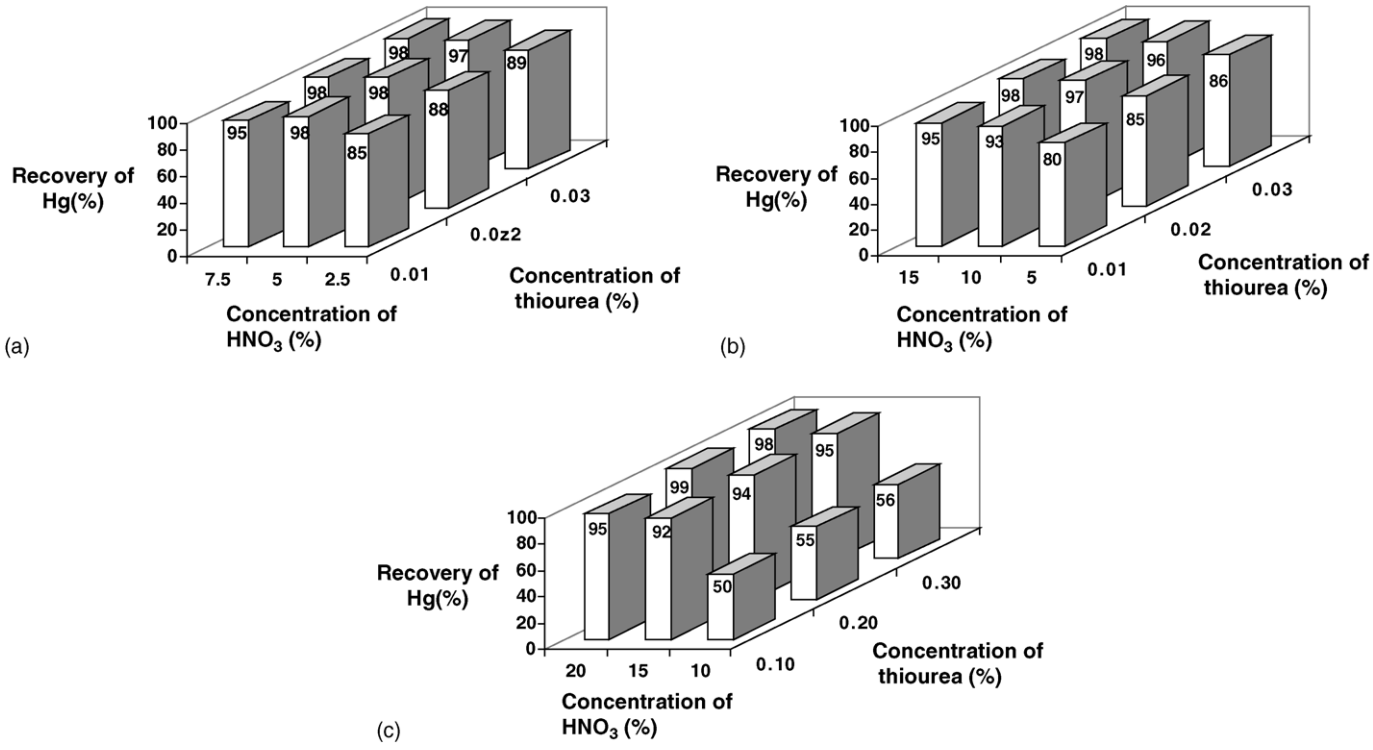

Fig. 1. (a) Optimization for quantitative recovery of total mercury from IAEA-350 (b) M-IM–MM and (c) NIST-1633b.

tative extraction of both the mercury species from fish tissues, plant samples as well as coal fly ash are shown in Fig. 1a–c, respectively. In all the cases, the extraction efficiency of mercury increased with acid concentration. Subsequent to sonication, total mercury was determined after converting MM to IM by UV irradiation.

3.1.1. Fish homogenate (IAEA-350)

As seen in Fig. 1a, the extraction efficiency of mercury increased with increasing HNO_3 and thiourea concentration; an extractant mixture of 5% HNO_3 and 0.02% thiourea yielded the best extraction (>95%) of both IM and MM species from fish tissues. The method was further validated by the analysis of additional certified reference materials: NRCC-DORM2 (Dogfish muscle) and NRCC-DOLT-1 (Dogfish liver) and the results are presented in Table 2. In all the cases, the difference between total and inorganic mercury was taken as MM, which is well in agreement with the certified values.

3.1.2. Mercury loaded moss

After equilibrating the mixture, the moss was separated by centrifugation and supernatant was analysed for residual mercury. The residual mercury in supernatant was found to be about 3–4% of the initial mercury (5 μg). This study indicated that about 96% of 5 μg had been taken up by moss.

As shown in Fig. 1b, in the case of mercury loaded moss (M-IM–MM), an extractant mixture of 10% HNO_3 and 0.02% thiourea was required for quantitative extraction of mercury species (IM and MM). A similar method of sonication was applied on IAEA-336 and the results indicated that the value obtained from the proposed sonication method was in good agreement with the certified value. So far the reported methods for plant samples were mainly based on wet ashing with conc. HNO_3 and microwave-assisted decomposition of the matrix using concentrated acids for the determination total mercury only [61]. Very few methods have been reported for the speciation of mercury in lichens and mosses [54,62].

Table 2
Analytical results obtained for fish tissues with the proposed ultrasound-assisted thiourea extraction method ($n = 5$)

Reference material code	Certified values (μg g^{-1})		Obtained in this work (μg g^{-1})			MW digestion
	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+ [a]	Hg^{2+}	Total Hg
IAEA-350	4.68 ± 0.28	3.65 ± 0.35	4.45 ± 0.26	3.53 ± 0.24	0.92 ± 0.06	4.59 ± 0.31
NRCC-DORM2	4.64 ± 0.26	4.47 ± 0.32	4.55 ± 0.22	4.32 ± 0.25	0.23 ± 0.03	4.65 ± 0.33
DOLT1	0.225 ± 0.037	0.080 ± 0.011	0.218 ± 0.028	0.075 ± 0.007	0.143 ± 0.013	0.219 ± 0.016

5% HNO_3 + 0.02% thiourea as extractant mixture; sonication amplitude = 40%; sonication time = 4 min.
[a] Values calculated as difference between total mercury and inorganic mercury.

Table 3
Analytical results obtained for mercury loaded moss and field samples (lichens and mosses) with the proposed ultrasound-assisted thiourea method ($n = 5$)

Sample code	Loaded values ($\mu g\,g^{-1}$)		Obtained in this work ($\mu g\,g^{-1}$)			MW digestion
	Hg^{2+}	CH_3Hg^+	Total Hg	CH_3Hg^{+a}	Hg^{2+}	Total Hg
M-IM	4.7 ± 0.2	–	4.5 ± 0.3	–	4.5 ± 0.2	4.8 ± 0.5
M-MM	–	4.6 ± 0.3	–	4.3 ± 0.2	–	4.5 ± 0.6
M-IM–MM	2.3 ± 0.2	2.4 ± 0.1	4.6 ± 0.4	2.3 ± 0.2	2.3 ± 0.1	4.7 ± 0.4
IAEA-336	0.2[b]		0.19 ± 0.01	–	–	0.21 ± 0.03
Lichen-1	–	–	7.1 ± 0.6	ND	7.1 ± 0.4	7.4 ± 0.8
Lichen-2	–	–	0.18 ± 0.02	ND	0.18 ± 0.02	0.19 ± 0.02
Lichen-3	–	–	0.07 ± 0.01	ND	0.07 ± 0.01	0.08 ± 0.01
Moss-1	–	–	5.5 ± 0.08	ND	5.5 ± 0.4	5.7 ± 0.6
Moss-2	–	–	0.16 ± 0.02	ND	0.16 ± 0.02	0.17 ± 0.03
Moss-3	–	–	0.06 ± 0.01	ND	0.06 ± 0.01	0.07 ± 0.01

M-IM, M-MM: laboratory reference material (moss) loaded with known amount of inorganic and methyl mercury, respectively. Lichen-1, 2, 3 and moss-1, 2, 3 are field samples collected near thermometer factory, 0.5 km away from factory and 15 km away from factory, respectively. ND: not detected. 10% HNO_3 + 0.02% thiourea as extractant mixture; sonication amplitude = 40%; sonication time = 3 min.

[a] Values calculated as difference between total mercury and inorganic mercury.

[b] Certified value with confidence interval 0.16–0.24.

Using the proposed sonication method, various lichen and moss samples collected at different distances from a thermometer factory (presently not in operation) located in Kodaikanal, Tamilnadu, a Southern state of India have been analysed. Sampling and sample preparation procedure have been described in detail elsewhere [52]. From Table 3, it may be seen that no significant quantity of MM was found in the collected lichen and moss samples. Similar observations were noticed in our earlier studies [52]. The proposed method is very fast and useful for the determination of mercury and its species in plant-derived samples.

3.1.3. Coal fly ash (NIST 1633b)

As can be seen from Fig. 1c, quantitative leaching of total mercury from coal fly ash was achieved with an extractant mixture of 20% HNO_3 and 0.2% thiourea. As the content of total mercury in NIST 1633b is low (141 ng g^{-1}) and also to avoid possible matrix interferences, the total mercury concentration of coal fly ash was determined by both external calibration and standard addition methods. The samples were spiked with known amount of IM (125 and 250 ng absolute) before ultrasonic extraction. Quantitative recovery (>95%) of spiked IM was achieved using the extractant mixture of 20% HNO_3 and 0.2% thiourea.

The effect of thiourea concentration on the relative recovery of mercury signal with respect to signal obtained in the absence of thiourea using $NaBH_4$ and $SnCl_2$ as reducing agents is shown in Fig. 2. As seen from Fig. 2, mercury signal recovery obtained with $NaBH_4$ was constant even when the solution contained 0.1% of thiourea whereas a significant decrease was noticed with $SnCl_2$ when the thiourea concentration exceeded 0.4%. Hence, only $NaBH_4$ was used as reducing agent for the determination of total mercury in subsequent sonication experiments with coal fly ash and coal samples.

A coal fly ash sample collected from a thermal power plant (Vijayawada, Andhrapradesh, India) and five coal samples obtained from different mines in India, were analysed for total mercury using the proposed ultrasound thiourea method with the optimized extractant mixture of 20% HNO_3 and 0.2% thiourea and the results are presented in Table 4.

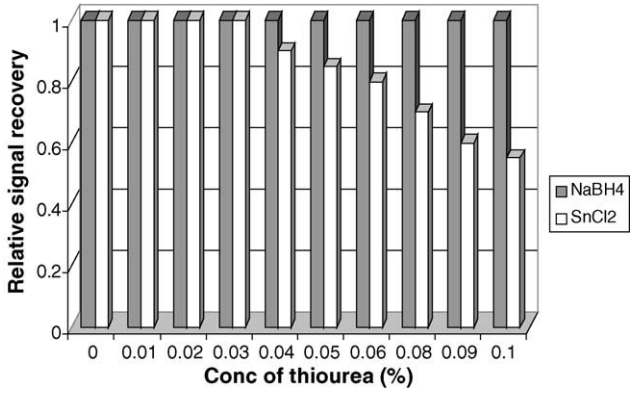

Fig. 2. Effect of concentration of thiourea on the recovery of mercury signal when $NaBH_4$ and $SnCl_2$ are used as reducing agents.

Table 4
Total mercury values obtained for Coal fly ash reference materials and field samples with the proposed ultrasound-assisted thiourea extraction method ($n = 10$)

Sample code	Certified values ($ng\,g^{-1}$)	Obtained in this work ($ng\,g^{-1}$)
	Total Hg	Total Hg
NIST-1633b	141 ± 19	135 ± 16
Coal fly ash field sample	–	125 ± 13
Coal sample-1	–	132 ± 12
Coal sample-2	–	176 ± 15
Coal sample-3	–	121 ± 12
Coal sample-4	–	134 ± 13
Coal sample-5	–	128 ± 15

20% HNO_3 + 0.2% thiourea as extractant mixture, sonication amplitude = 40%; sonication time = 4 min. Coal fly ash field sample was collected at a thermal power station. Coal samples were obtained from different coalmines in India.

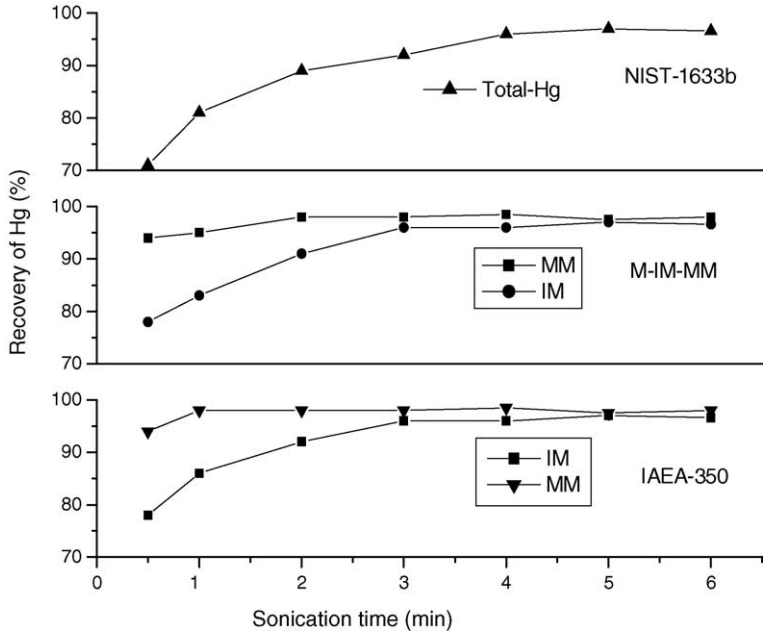

Fig. 3. Effect of sonication time on IM and MM extraction from (a) IAEA-350 (extractant 5% HNO_3 + 0.02% thiourea) (b) M-IM–MM (extractant 10% HNO_3 + 0.02% thiourea) and (c) NIST-1633b (extractant 20% HNO_3 + 0.2% thiourea), sonication amplitude 40% was used in all cases.

When the similar sonication experiments were carried out with fish tissues, mercury loaded moss and coal fly ash, similar results were obtained with HCl in lieu of HNO_3 or even with mixture of HCl and HNO_3 used as extractants. In all the above studies, the recoveries of inorganic and total mercury were quantitative (>95%).

3.2. Effect of sonication time

The effect of change of sonication time on recoveries obtained for IM and MM after sonication using 5% HNO_3 + 0.02% thiourea (IAEA-350), 10% HNO_3 + 0.02% thiourea (M-IM–MM) and 20% HNO_3 + 0.2% thiourea (NIST-1633b) as extractant solution is shown in Fig. 3. In all the samples, extraction efficiency of IM and MM increased with increasing sonication time from 1 to 4 min and plateaued thereafter.

Choosing sonication amplitude of 40%, a sonication time of 3 min (4 min in case of NIST 1633b) and the sample weight of 100 mg (200 mg in the case of NIST 1633b) the best recoveries for both IM and MM could be achieved. The results clearly indicate that sonication time of 4 min was sufficient for the quantitative extraction of both the forms of mercury from mercury loaded moss, fish homogenate and coal fly ash samples, which is advantageous for high sample throughput.

3.3. Effect of sonication amplitude

Taking optimum extraction times obtained at each stage, a study of the influence of sonication power on recovery of Hg has been made. The effect of ultrasound amplitude on extraction of mercury from lichen sample in the range 20–80% is shown in Fig. 4 keeping the remaining variables such as extractant concentration, sonication time and sample weight at values fixed above. The extraction efficiency increased with increasing amplitude from 20 to 40% for fish tissues and plant samples) and 20–50% in case of coal fly ash and remained constant at higher amplitude values.

It is known that intensity of ultrasound transmitted to the medium is directly related to the vibration amplitude of the probe. However, at very high vibrational amplitude, a great number of cavitation bubbles are generated in the solution, which may dampen the passage of ultrasound energy through the liquid. The curve obtained for IM and MM indicates that 40% amplitude is required for quantitative recovery after extraction whereas the extraction efficiency did not improve at higher ultrasound amplitude. Hence, a sonication amplitude of 40% for fish tissues and plant samples whereas 50% for coal fly ash was used in all the subsequent experiments. As observed by earlier authors [45,54], the variation in the recovery of MM was less pronounced than that of IM in fish tissues and plant samples.

3.4. Stability of Hg species after sonication

The methyl mercury stability during ultrasound-assisted extraction was studied using M-MM. At all extraction conditions, IM was determined in extracts using $SnCl_2$ as reducing agent. This study revealed that the sample M-MM did not contain detectable amounts of inorganic mercury and moreover, decomposition of MM to IM did not occur during sonication. Similar observations were noticed by Rio-Segade and Bendicho [45] in studies related to ultrasound-assisted extraction for mercury speciation in fish tissues using HCl as extracting

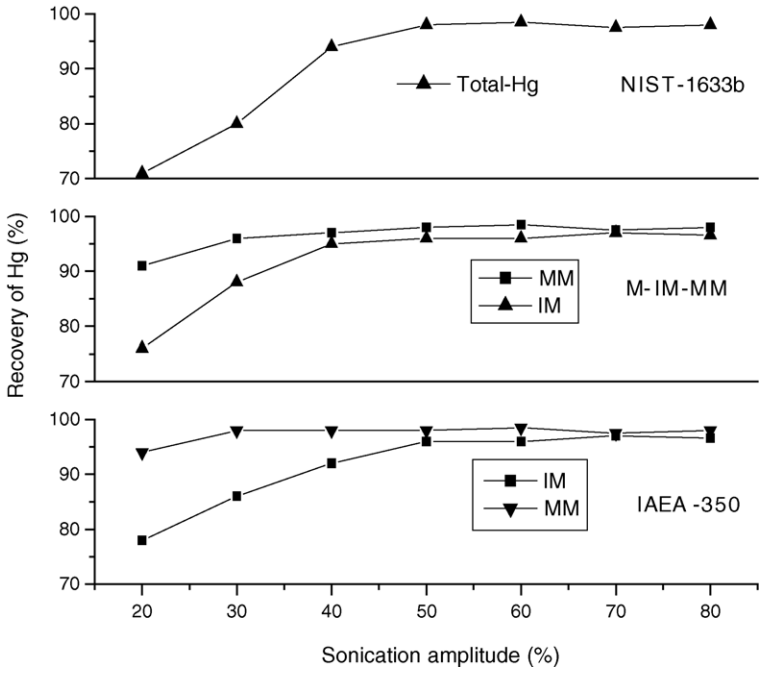

Fig. 4. Effect of sonication amplitude on IM and MM extraction from (a) IAEA-350 (extractant 5% HNO_3 + 0.02% thiourea) (b) M-IM–MM (extractant 10% HNO_3 + 0.02% thiourea) and (c) NIST-1633b (extractant 20% HNO_3 + 0.2% thiourea). Sonication time 3 min (for animal tissues and plant samples) and 4 min (for coal fly ash) was used in all cases.

agent. After sonication and centrifugation, the stability of the IM and MM species was also checked with respect to time by analyzing the supernatant at different time intervals. These studies showed that the species are stable in the supernatant even after 24 h of sonication.

3.5. Oxidation behaviour of MM

After sonication, the extracted species were oxidized to IM by UV irradiation. The oxidation recovery of MM obtained with UV irradiation is shown in Fig. 5. The principal variable associated with MM, which could influence the oxidation is acidity, i.e., HNO_3 concentration which was varied in the range of 0–10%. Five millilitres of aqueous solution was passed in each run by keeping concentration of MM (100 ng/ml) constant. Similar experiments were carried out in the presence of 0.02% thiourea, an optimized concentration in our sonication experiments (for fish tissues and plant samples).

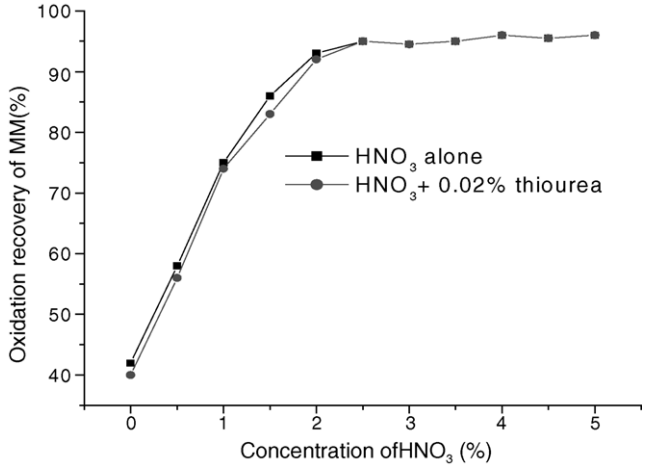

Fig. 5. Effect of concentration of nitric acid on the oxidation recovery of methyl mercury.

As seen from Fig. 5, quantitative oxidation of MM ($94 \pm 5\%$) was observed when the sample solution contained >2% of HNO_3. Similar observations were noticed when the sample solution contained 0.02% of thiourea which is the optimum concentration used in the extraction studies of animal tissues and plant samples. This behaviour could possibly be due to the formation of various free radicals (OH, NO_2, NO_3, etc.) which enhances the oxidation of MM. This has to be further investigated in detail.

3.6. Analytical figures of merit

The whole analytical procedure proposed for the determination of IM and MM in fish tissues, samples of plant origin and coal fly ash is presented schematically in Fig. 6. The performance of this procedure has been evaluated by the analysis of IAEA-350, DORM-2, DOLT-1, IAEA-336 and NIST-1633b materials. The results corresponding to various reference materials are in good agreement with the certified values. Based on the blank measurements, the limit of detection (LOD) values were 15 and 10 ng g^{-1} for MM and IM, respectively. The proposed ultrasound-assisted extraction procedure significantly reduces the time required for

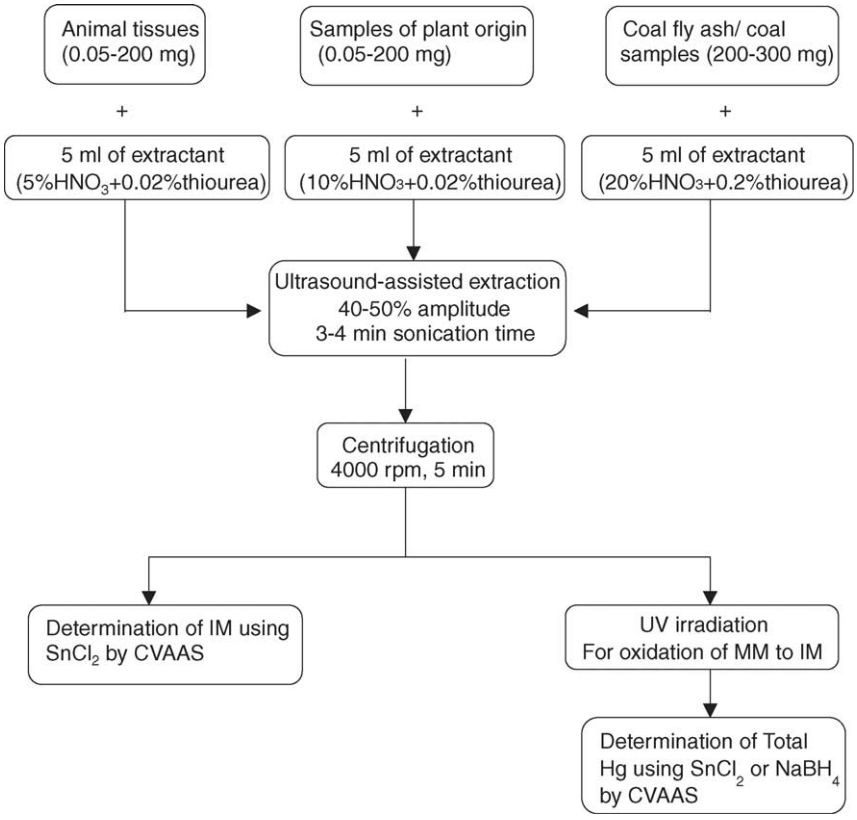

Fig. 6. Schematic flow diagram of the proposed ultrasound-assisted thiourea extraction method for the analysis of total mercury and its species from various matrices.

sample treatment for the extraction of Hg species. The average time required for the analysis of each sample using the proposed extraction method is 15–20 min while the conventional solvent extraction methods [20,21] take about 4 h, acid slurry sampling methods [63] require about 24 h, extraction times of about 90 and 30 min are required for distillation [21] or supercritical fluid extraction procedures [25]. In addition, keeping the number of analytical steps to a minimum considerably reduces the source of analytical errors.

4. Conclusions

A simple and rapid ultrasound-assisted thiourea extraction method for the determination of total mercury and its species in biological and environmental samples is presented. Quantitative recovery of total Hg was achieved using a mixtures of 5% HNO_3 and 0.02% thiourea, 10% HNO_3 and 0.02% thiourea and 20% HNO_3 and 0.2% thiourea for animal tissues, plant matrices and coal fly ash samples, respectively. The number of analytical steps involved is minimum and lowers the contamination problems. The obtained results were in close agreement with certified values with an overall precision of better than 5–15% in all the cases. The proposed method was applied for the determination of Hg in real samples of lichens, mosses, coal fly ash and coal samples.

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